JOURNAL OF AGRICULTURAL RESEARCH

WASHINGTON, D. C., DECEMBER 22, 1923. No. 12.

CYTOLOGICAL STUDIES OF INFECTION OF BAART, KANRED, AND MINDUM WHEATS BY PUCCINIA GRAMINIS TRITICI FORMS III AND XIX[1]

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INTRODUCTION

The recent discovery of the specialized or so-called biologic forms of wheat stem rust (*Puccinia graminis tritici* Erikss. and Henn.) and the realization of the importance of this discovery in the work of combating the disease have led to intensive study of the problem. The knowledge that we are not dealing with one uniform fungus, but with numerous strains morphologically similar but physiologically distinct, and each with its own limited range of power of infection, complicates seriously the problem of breeding for rust resistance.

Stakman and Piemeisel (*40*), [3] in 1917, published an account of a second strain of wheat stem rust; Levine and Stakman (*21*), in 1918, reported a third; and Melchers and Parker (*25*), in 1918, still another. Extensive work then was undertaken by Stakman and his associates to "determine the number, characteristics, and distribution of biologic forms, as well as their constancy and probable origin." In a preliminary report, in 1919, Stakman, Levine, and Leach (*38*) reported about a dozen specialized or biologic forms of wheat stem rust; and, in 1922, Stakman and Levine (*37*) reported 37 such forms differing in their power to infect the varieties of wheat chosen as differential hosts. They give a key by which these specialized rust forms may be identified.

Specialized forms of stem rust have been under observation too short a time to make it certain that they are permanent stable entities, but such data as have been reported on the six main subdivisions of *Puccinia graminis* by Stakman and Piemeisel (*41*) and on the specialized forms within *Puccinia graminis tritici* by Stakman, Piemeisel, and Levine (*42*) and Stakman, Parker, and Piemeisel (*39*) show that they do not change their infecting power readily and are probably to be considered as permanent independent strains of the fungus.

The students of cereal rusts are agreed that rust resistance is due to one or more hereditary factors that behave in Mendelian fashion. Nilsson-Ehle (*28*), Biffen (*6*), and Armstrong (*3*), working on stripe rust of wheat; Parker (*29*), on crown rust of oats; Garber (*13*, *14*) and Griffee (*16*), on stem rust of oats; and Waldron (*43*), Puttick (*30*), Aamodt (*1*), Melchers and Parker (*26*), and Hayes, Parker, and Kurtzweil (*18*), in their study of the stem rust of wheat, all believe that "the technique of breeding for rust resistance is similar to that of breeding for agronomic characters."

[1] Accepted for publication August 11, 1923. Cooperative investigations between the Agricultural Experiment Station of the University of California and the Bureau of Plant Industry, United States Department of Agriculture.

[2] The writer makes grateful acknowledgment to Dr. H. B. Humphrey, at whose suggestion the research was undertaken, for steady encouragement during the work, and to Professor W. W. Mackie and Professor E. B. Babcock for the numerous courtesies extended during the progress of the study.

[3] Reference is made by number (italic) to "Literature cited," pp. 602–604.

In the more recent work on breeding for resistance to stem rust in wheat, account has been taken of the physiologically distinct forms of the rust, and specific rust strains have been used. The relations between the various rusts and hosts are not uniform.

Hayes, Parker, and Kurtzweil (*18*), using a known strain of stem rust, find that "in the cross where Emmer is one parent, resistance is partially dominant, while in the Durum-common cross, susceptibility is completely dominant over resistance."

Puttick (*30*) studied the infection results of forms I and XIX of *Puccinia graminis tritici* on a cross between Mindum and Marquis. Mindum is immune from I and susceptible to XIX, while Marquis shows the reciprocal relation. In the F_2 inoculated with form XIX there was evidence of one main factor and other modifying factors concerned in rust resistance. The same plants exposed to form I show that several factors are involved.

Aamodt (*1*) and Melchers and Parker (*26*) studied the reaction of the hybrid Kanred × Marquis to known strains of stem rust. They agree that in this cross immunity is dominant and that there is a clean-cut 3 to 1 ratio in the F_2. Aamodt states further that in this particular cross a single factor determines the reaction to several specialized forms of the rust.

Little is known concerning the relationship of rust and host in these different reactions. Resistance to the rust evidently depends on different factors in different cases. The differences between hosts and also between rusts, in some cases, may be differences in degree rather than in kind. The work of Hursh (*19*) demonstrates distinct differences between two biologic forms of the rust as to tolerance of extremes of hydrogen-ion concentration and temperature.

A detailed cytological study of the behavior of rust and host in these various cases may give some insight into the nature of rust resistance. This paper is the second of a series undertaken in the hope of learning more concerning the nature of immunity. The earlier cytological studies of cereal rusts, by Eriksson (*11*), Ward (*44*), Pole Evans (*12*), Gibson (*15*), Marryat (*24*), and Stakman (*35, 36*), were reviewed in the first paper of this series (*2*).

INVESTIGATIONS

MATERIAL AND METHODS

The specialized forms of rust were III and XIX. Viable spores of form III were supplied by Doctor Stakman. The other rust was found at Berkeley on wheat growing in the botanical garden of the university. It was used in former studies (*2*) of infections of Baart and Kanred under the name of Berkeley rust, and has since been identified by Mr. M. N. Levine as form XIX. As before, the varieties of wheat used were Baart, seed for which was grown in the cereal plots at Davis, Calif., in 1919; Kanred (C. I. No. 5146), received from Hays, Kans., in 1917; and Mindum (C. I. No. 5296, Minnesota No. 470).

The following are the rust relationships, using a scale of 0 to 4, in which 0 represents complete immunity, and 4, great susceptibility:

	TYPE OF INFECTION	
	Form III	Form XIX
Baart	4	4
Kanred	3	0
Mindum	0	3

Seedlings were grown in the greenhouse in 4-inch pots. About 10 days after sowing, the first leaf of each seedling was inoculated, the plants placed in damp chambers 48 hours and then kept in cheese-cloth cages. Material was fixed daily from the second to the fifteenth day after inoculation and, for comparison, different lots were grown and fixed at different times of the year.

On the whole, the best fixation was obtained with the standard solution: 1 gram chromic acid, 1 gram acetic acid, ½ gram urea, in 100 cc. of distilled water. The chief trouble in fixation seems to be due to slow penetration. The majority of the stomata in wheat are closed, and the rest of them close partly or completely in the fixing fluid. The air imprisoned in the large intercellular spaces of the leaf is displaced but slowly, if at all, by the fixing fluid. The fluid works its way in only through the cut surfaces at the ends of the piece. The spongy mesophyll tissue composing the greater part of the interior of the leaf is made of lobed, irregular cells having small surfaces of contact with each other. Penetration from one to another of these cells is slow, and the central tissues of a piece of leaf deteriorate, especially in a warm room, before the fixing fluid reaches them. This trouble was largely obviated by placing material during fixation in a cold-storage chamber at 42° F., which preserved the tissues during penetration. The tissues were embedded in paraffin, sectioned, and stained with Flemming's triple stain.

BAART AND PUCCINIA GRAMINIS TRITICI FORM III

MACROSCOPIC OBSERVATIONS

Form III of *Puccinia graminis tritici* Erikss. and Henn. produces the 4− or 4 type of infection on seedlings of Baart wheat. It develops vigorously and gives evidence of being on a congenial host. The flecks or discolorations of the leaf marking the location of the young infections make their first appearance about the sixth day after inoculation. These are ellipsoidal in shape, a uniform light green in color, and about a millimeter in diameter. The flecks grow, and on the eighth or ninth day the fungus breaks through the epidermis at the center of each spot, forming the open uredinium. At maturity, about two weeks after inoculation, the uredinia, if isolated, are 3 or 4 mm. long and about half as wide and are surrounded by a zone of paler tissue 1 to 2 mm. wide. This discolored area is not white or gray or yellow, but is green and only a shade or so lighter than the rest of the leaf. When uredinia are crowded together, they are much smaller and the discoloration of the leaf may be continuous.

MICROSCOPIC OBSERVATIONS

An earlier paper (2) reports a cytological study of Baart infected with *Puccinia graminis tritici* form I and another strain of stem rust found in Berkeley and since identified as form XIX. Baart is susceptible to both of these forms of the rust, and their history was followed in some detail through the first week of development, giving the formation of the appressorium upon the stoma, its entry through the stomatal slit, the formation of the substomatal vesicle, the growth of the infecting hyphæ from it, the formation of haustorium mother cells, and the development of haustoria. *Puccinia graminis tritici* III grows equally freely and normally on Baart. A description of the first week of its development would be in large measure a duplication of what already has been written, and so is not repeated here.

Entrance Phenomena

One or two points, however, should be noted. The urediniospores of form III germinate readily and appressoria are formed on the stomata. Usually the fungus passes between the guard cells promptly. In some cases the entry is delayed, and in others the fungus fails to enter. Appearances are normal for the first three days. On the fourth day, however, several cases have been observed in which the guard cells of the occupied stoma show slight signs of deterioration. This reaction is not general, at least half of the stomata visited by fungi remaining unaffected, but when it does occur, it is equally likely to happen whether the fungus has entered or not.

Normally the heavily thickened wall of a guard cell has a strong affinity for both the safranin and the gentian of the triple stain. In fact, the purple-stained wall is so nearly opaque that it is sometimes difficult to see the cell contents. At this stage, in a few cases, the part of the guard cell wall in immediate contact with the fungus has undergone some chemical change. It no longer takes any stain, but is transparent and glistens conspicuously. At first only a small area under the center of the appressorium shows this change; later the whole surface in contact with the fungus is colorless, and still later a part of the inner wall opposite the appressorium is affected.

Plate 1, A, represents a longitudinal section of a stoma showing one of the two guard cells with the appressorium *a* on its outer surface. The plant was grown and inoculated in August, 1922, and fixed seven days after inoculation. The appressorium is pale and dying and its nuclei are no longer distinguishable, for appressoria wither quickly at summer temperatures. The guard-cell wall in contact with the appressorium has lost power to stain and part of the inner wall *c* is pale. The contents of this part of the cell also are affected. The guard-cell nucleus has normally the form of an elongated dumb-bell. Here the thin connecting strand of nuclear material has been dissolved at *b*. The two ends of the cell are still living. In a few cases the reaction is even more pronounced and the entire cell is dead.

A question arose as to whether weather conditions could affect this reaction. Three lots of seedlings grown in October, 1921, and in March and August, 1922, and fixed six or seven days after inoculation, were studied and compared. The stomata of the three lots of material proved to be very similar, showing little or no effect of the varying light, temperature, and moisture at different times of the year. Of the 68 cases recorded, 36 showed no change due to the fungus, 28 were more or less harmed, and only 4 were completely killed.

Apart from this reaction on the guard cells, the host and fungus seem to be congenial. The fungi which enter develop vigorously.

Development of the Fungus After Entrance

The period between the entry of the fungus and the sixth day after inoculation is one of intensive vegetative development in the green tissue of the leaf. This spongy mesophyll tissue is composed of large irregular much lobed cells. Beneath each stoma is a large intercellular space, the substomatal chamber, and this is in communication with numerous smaller air spaces all through the tissue. More than half of the volume of this tissue is in the intercellular spaces. Hyphae with rich cytoplasm and large nuclei follow the surfaces of the mesophyl

cells, branching freely and forming numerous haustoria. Within a limited area in the leaf, perhaps a millimeter in diameter, each chink and cranny of intercellular space becomes filled with the fungus, which forms little masses of pseudoparenchyma conforming closely to the shape of the irregular passages they occupy. The host cells retain their shape and spatial relations, and in spite of the fact that the fungus is choking the air passages around them and forming numerous haustoria which absorb the food within them, these host cells are still living and fairly vigorous. Haustoria are formed in abundance and expand freely, growing into long worm-shaped bodies sometimes extending across the cell.

When the fungus is well established in its host and is rich in protoplasm and food, it enters upon a new phase marked by two activities, the formation of uredinia and the sending out of long stolon-like hyphae to fresh areas in the leaf tissue. Both of these activities are aided by a translocation of food materials through the hyphae from the center of the mycelial mass to its periphery. The method by which this transportation takes place is not obvious. The hyphae are septate, being composed of relatively short binucleate cells. The septa are not porous, so far as could be determined, or if they are, the pores are ultramicroscopic in size. Yet, in some fashion, the contents of the hyphae, and to some extent even those of the haustoria, move out to the regions of active growth.

Haustoria in full vigor contain apparently dense granules scattered through the cytoplasm. In infections about a week old, the granules begin to disappear, then much of the cytoplasm follows, leaving the haustorium nearly transparent. Something very similar in appearance occurs in the hyphae of the whole central region of the infection. Food granules and cytoplasm disappear gradually, leaving the hyphae nearly empty. Plate 1, B, shows a bit of intercellular mycelium at *b*, and a haustorium at *a*, from an infection a week old. The haustorium is pale and its contents scant, and the hyphae *b* outside of the cell are nearly empty. Another change is in the haustorium mother cell, which hitherto has been clear, empty, and thin-walled. Now (pl. 1, B, at *c*) the haustorium mother cell presents an appearance suggesting that the walls, or at least the inner lamellae of the walls, are swelling. The swollen wall, if such it is, takes a faint pink stain and is glassy in appearance. This change varies greatly in different lots of material, and, in extreme cases, as in plate 1, C, at *a*, and D, at *a* and *b*, the lumen of the haustorium mother cell may be almost obliterated.

As the haustoria become transparent, due to the disappearance of the granules, there frequently are revealed within them one or two rounded bodies, strongly resembling nuclei. These may be the nuclei of the original cell which contributed its contents to form the haustorium. These are to be seen clearly in Plate 1, C, at *b*, in D at *c* and *d*, and in E at *a*.

A minor part of the food drained from the central mycelium is utilized by the stolon-like hyphae. Unlike the earlier hyphae, these "stolons" do not feel their way along the surface of the cells, conforming closely to their irregularities, nor do they form a haustorium whenever the tip strikes against a host cell. These well fed, rapidly growing, sparsely septate stolons strike out away from the center of infection, growing across the intercellular spaces as straight as the irregular passageways of the leaf permit. When they reach fresh tissues, they start new

centers of infection, which result ultimately in a circle of secondary uredinia around the first. Similar stolons occur in *P. glumarum* (Schm.) Erikss. and Henn., as described by Pole Evans (*12*, *p.* 452), and in *P. dispersa* Erikss., as described by Ward (*44*, *p.* 37).

The greater part of this surplus food, however, is utilized in spore formation. By the seventh day, at the center of the infection, hyphae are massing under the epidermis antecedent to the formation of uredinia. In Plate 1, F, is shown a portion of a young uredinium, drawn on a smaller scale than the preceding figures. It happens here that the uredinium is forming under the same stoma through which the fungus entered. The old collapsed wall of the appressorium is still visible at *a*. In this case the guard-cell wall was but slightly affected by the fungus, there being a small pale spot on the inner wall below the appressorium. There is a felt of hyphae filling the intercellular spaces and running up into the substomatal chamber where the young binucleate spores are forming. The hyphae at the center of the infection and even up near the uredinium are well drained of their contents; in fact, are nearly empty, and the food is now concentrated in the spores and the layer of hyphae just below them.

As growth proceeds, the young spores lift the epidermis, rupture it, and both the spores and their stalks elongate. In Plate 1, G, is represented a narrow strip through the center of an infection two weeks old. Spore formation is taking place on both surfaces of the leaf, *a* and *b*. There is a massive development of mycelium just below the surface, and both here and in the central region the mycelium is devoid of contents. Numerous spores have already been freed, as can be determined from the withered stalks that remain. A few fresh spores are still being formed. Some of the host cells are crowded out of shape and almost obliterated, but in many cases are still living.

In older infections there were noted for the first time a few scattered haustoria which had the appearance of having formed in an uncongenial environment. They were only partly expanded and were surrounded by a thick, glistening, transparent covering. It may be that they indicate some feeble and tardily developing resistance to the rust which shows in but a small percentage of the host cells. The rest of the haustoria looked normal even when the contents of the host cell had collapsed.

Effect of Neuclei and Plastids of Baart

This luxuriant growth of the fungus takes place with the minimum of harm to the host, but a close comparison of successive stages shows that the host tissues within range of the fungus undergo a fairly definite series of metabolic changes. The most tangible evidence of this is the alteration in size and shape of the host nuclei and plastids.

The nuclei in the affected area expand rapidly, remain large for several days, and then collapse and die. Typical stages in this process are represented in Plate 2, A, *a* to *h*. The same magnification (× 1130) was used throughout the series. The normal nucleus, *a*, uninfluenced by the fungus, has a heavy membrane, a relatively small nucleolus and a nuclear net which is usually very dense but occasionally shows open places. When the fungus is six or seven days old, nuclei of the mesophyll cells in the center of the infection begin to expand. The nuclear net opens irregularly, forming a larger mesh along one side (Pl. 2, A, *b* and *c*), as if the nucleus were taking in water on this side. Nucleolar material

also is increasing in quantity. This expansion continues (*d* and *e*) until the volume is several times the normal and the thin delicate nuclear membrane incloses a large meshed chromatin net composed of very fine threads. The nucleolus is greatly enlarged. This condition is maintained for only a few days and is followed by the collapse and death of the nucleus (*f* and *g*). This may begin on the tenth day but usually is not conspicuous until a little later. By the fourteenth day, however, the nucleus is flattened into a disk, usually lying against the cell wall. This decrease in volume is best seen in edge view (*h*). The nucleus is now a red stained body from which all trace of nuclear structure has been lost.

To obtain more exact information as to the extent of the nuclear changes in an infection area and beyond it during the spread and development of the fungus, outline camera drawings were made of nuclei in the center of the infection and at graded distances from it. In each zone in infections 7, 10, and 14 days of age, 10 nuclei were drawn (× 1130) and both their long diameters and short diameters were measured and added. The figures in the tabulated results (Table I) are not in microns but are directly comparable, as each represents the sum in millimeters of the long and the short diameters of 10 nuclei or 10 plastids at a magnification of 1130.

The figures for the normal nucleus are about 115 by 70. In the maetrial from seven-day infections the effect of the fungus is pronounced, the nuclear size at the center of the infection being 140 by 90. The effect of the fungus is seen only at the center of the infection in the earlier stages, but soon extends farther outward, keeping pace to some extent with the spread of the fungus itself.

The maximum nuclear size is found in ten-day infections (Table I, A, 10a and 10b), where the nuclei in the vicinity of the young uredinium reach the dimensions 143 by 116 and 159 by 123, showing that the volume of the nucleus has increased several fold. There is less and less enlargement of the nuclei toward the margin of the infection area and at this stage there is little, if any, change from the normal nuclear condition beyond the range of the fungus itself.

Four days later (14a), when spore formation is at its height, the host nucleus collapses, as is shown by the sharp decrease in the short diameter, which is now reduced from 100 or even more to about 40. Moreover, the dimensions of nuclei in the area which is beyond the fungus, 145 by 49, show that here, too, the nuclei undergo a tardy expansion and then collapse.

It is of interest to note the effect where two uredinia occur just far enough apart so that their mycelia are tangent, or nearly so. In this case (Table I, B, nuclei, 10c, 14b) the nuclei expand in cells throughout the intermediate area between the two uredinia.

A different relation exists between the fungus and the size of plastids in infected host tissue. Measurements here (Table I, plastids) were made from the same areas of the same infections that were used in the nuclear studies and the same methods were followed.

In general the plastids within the infected region are markedly decreased in size. The numbers for the normal plastid (Table I, A, plastids, 2a) are 60 by 25. One week after inoculation (7a and 7b) plastids throughout the infected area are reduced one-third or more, being approximately 40 by 20. With few exceptions the plastids in the center of the infection show no further reduction, maintaining a fairly constant size somewhere near 40 by 20 until the uredinium is full grown.

TABLE I.—*Comparative size of host nuclei and of plastids in or near infections of Puccinia graminis tritici form III of different ages on seedlings of Baart wheat* [a]

A. TISSUE IN OR SURROUNDING AN INFECTION

Days after inoculation.	Center of infection.	Center of uredinium.	Margin of uredinium.	Margin of infection.	Adjacent uninfected tissue.	Normal or nearly normal tissue.
HOST NUCLEI						
2a	.....	.....	.....	.....	.....	115×70
7a	140×90	.....	.....	129×91	102×70	111×80
7b	136×98	.....	.....	135×94	114×84	113×68
10a	.....	143×116	159×123	110×84	113×71	113×90
10b	.....	126×75	128×109	107×82	107×82	113×82
14a	.....	141×42	120×38	146×43	145×49	110×80
PLASTIDS						
2a	.....	.....	.....	.....	.....	60×25
7a	38×17	.....	.....	43×23	52×25	57×25
7b	41×26	.....	.....	40×21	53×24	60×26
10a	.....	44×27	32×26	25×14	22×15	40×25
10b	.....	28×20	33×23	20×14	27×20	41×21
14a	.....	37×20	45×24	39×24	41×23	46×20

B. TISSUE IN AND BETWEEN TWO NEIGHBORING UREDINA

Days after inoculation.	Center of first uredinium.	Margin of first uredinium.	Mycelium somewhat sparse.	Midway (very few hyphæ).	Mycelium somewhat sparse.	Margin of second uredinium.	Center of second uredinium.
HOST NUCLEI							
10c	148×115	127×115	127×112	117×98	124×111	142×115	142×117
14b	142× 45	169× 48	150× 53	148×58	136× 56	136× 51	140× 56
PLASTIDS							
10c	34×22	35×21	30×21	31×24	26×17	37×25	40×25
14b	41×23	46×23	37×19	37×18	40×19	42×21	50×22

[a] Each figure given represents the sum in millimeters of the diameters of 10 nuclei or 10 plastids × 1130.

The history of the plastids in the outlying portions of the infection area is less simple. As early as the seventh day after inoculation the influence of the fungus has extended for a short distance beyond the tips of the outer hyphae. The reduction in plastid size is slight (52 by 25) but unmistakable. Ten days after inoculation (Table I, A, plastids, 10a and 10b) the plastids at the margin of the infection, 25 by 14, and a considerable distance beyond it, 22 by 15, are reduced in size far below those at the center of the same infection. Even at the end of the section, some

distance farther on, the plastids, though larger, are below normal. By the fourteenth day (14a), on the contrary, there is a partial recovery in size and there is no longer any zone of tissue with markedly decreased plastids. In fact, the plastids are now of about the same size throughout.

The area between two neighboring uredinia (Table I), where the influence of the fungus is felt from both sides, does not show the extreme reduction in plastid size at any stage in development, so far as observed.

KANRED AND PUCCINIA GRAMINIS TRITICI FORMS III AND XIX

MACROSCOPIC OBSERVATIONS

On Kanred, form III of *Puccinia graminis tritici* Erikss. and Henn. produces the 3 type of infection. It produces uredinia but, while there are no marked signs of uncongeniality, the growth is somewhat less vigorous than on Baart.

MICROSCOPIC OBSERVATIONS

Entrance Phenomena of Forms III and XIX

In an earlier study (2) of Kanred inoculated with forms I and XXI (then referred to as the Berkeley rust), from which it is immune, it was found that this variety excluded a large percentage of the fungi. Appressoria form on the stomata but many do not enter. A natural question in connection with the partial exclusion of the fungus by Kanred is whether congeniality or uncongeniality of the rust affects the entrance. A preliminary study of form III, which produces spores freely on Kanred' indicated that it, too, fails to enter Kanred in the majority of cases.

Further data have been taken on this point. One lot of seedlings was grown in July, 1921. In material fixed four days after inoculation, 9 fungi out of 82 had entered, or 11 per cent. An attempt was made to count some of this material fixed seven days after inoculation, but it was not satisfactory, as the weather had been exceptionally warm, and under greenhouse conditions the appressoria which did not enter withered quickly and some had fallen from the leaf.

In order to compare the two rusts on Kanred and eliminate as far as possible any differences that might be due to environment, a pot of Kanred seedlings was inoculated with form III and another at the same time (the latter part of April, 1922) with form XIX. These were given parallel treatment throughout. Material of each was fixed two, four, and six days after inoculation. The results are presented in Table II.

TABLE II.—*A comparison of forms III and XIX grown on Kanred, showing the percentages that have entered the host at different dates after inoculation*

Days after inoculation.	Form III, rust grade 3.			Form XIX, rust grade 0.		
	Number of—		Percentage of entries.	Number of—		Percentage of entries.
	Fungi.	Entries.		Fungi.	Entries.	
2	60	3	5	209	23	11
4	128	22	17	200	36	18
6	193	20	10	108	15	14
Total	381	45	12	517	74	14

The percentage of entries varies from 5 to 18 in the different lots. It is doubtful whether these differences are of great significance. The question is complicated by the fact that the percentage of entries may vary in different parts of the same leaf. On one part of a leaf 5 out of 73 entered, or about 7 per cent. On another part of the leaf, less than an inch away, 9 out of 59, or 15 per cent, entered. On one portion of another leaf, 5 out of 76, or 6½ per cent, entered, while on another portion 18 out of 133, or 13½ per cent, entered.

There evidently are local factors that influence the entry of the fungi. Taken as a whole, however, the evidence is fairly conclusive that so far as these two specialized forms of stem rust are concerned, the entry is not materially affected by congeniality or lack of it. The general average is 12 per cent of entries of form III, and 14 per cent of form XIX.

The guard cells of Kanred stomata undergo the same change when in contact with appressoria that was seen in Baart. In Plate 2, B, are seen two empty appressoria on a stoma, one guard cell of which was broken in sectioning. Both fungi have entered. The substomatal vesicles on the inner side of the stoma (*e* and *f*) have each produced an infecting hypha. These have grown in opposite directions and each in turn has formed a vigorous-looking haustorium in an epidermal cel l (*c* and *d*). The fungus looks vigorous and thriving, the epidermal cells invaded by haustoria show no disturbance, and the contents of the guard cells are living. The nucleus of one guard cell has shortened into a lump at *a*, but is otherwise normal in appearance. The guard cell walls facing the appressoria at *b*, however, have lost power to stain and are glassy in appearance. These altered walls are not appreciably swollen; in fact, neither here nor in the older infections by this rust on Kanred has any swelling of the cell walls been observed. The walls of the epidermal cells adjoining an occupied stoma are sometimes slightly modified but show very little swelling. In this same lot of material (taken four days after inoculation) all gradations can be found from healthy guard cells to those that are dead and colorless throughout.

Plate 2, C, is taken from material a week old. Both the appressorium and the guard cell are dead and disorganized and the adjoining epidermal cells weakened. The cell has broken from its support in sectioning. Stomata with guard cells killed and broken are much more common in this material than in Baart. An interesting point about this modification of walls exposed to the appressorium is that sometimes a small plate of wall material, as in the lamella in Plate 2, C, at *b*, will resist the attack and stain normally, although surrounded on all sides by perfectly colorless transparent walls.

Development of Form III After Entrance

The fungi which enter grow and develop in almost normal fashion. The hyphæ look well nourished and have good sized nuclei (pl. 2, E, at *a* and *b*), and they soon fill the intercellular spaces with a felt of interwoven threads. Haustoria form in the usual fashion and expand fully. In Plate 2, D, the haustorium mother cell at *b* has just begun to form a haustorium, *a*, within the host call. In Plate 2, B, at *c* and *d*, are seen half-grown haustoria. These appear to be covered by a rather heavy layer of host cytoplasm but are expanding freely nevertheless; and in E is a full-grown haustorium, *c*, connected by a narrow neck with the empty haustorium mother cell at *d*. This haustorium has extended across the

host cell and its contents have expanded into an open reticulum. The sheath of host cytoplasm inclosing the haustorium is not demonstrable in this case, but in material which has shrunken slightly, a thin clear space is to be found between the limiting osmotic membrane of the haustorium and an outer delicate sheath inclosing it. The cytoplasmic sheath covering young haustoria is continuous with the peripheral cytoplasm of the host cell and it seems probable that this outer covering of mature haustoria is derived from it.

Here, as in Baart, the sixth day marks a change in the activities of the fungus. Hyphae form long stolons which strike out through the intercellular spaces towards fresh areas of the leaf. In Plate 3, A, is represented a group of these stolons crossing a substomatal chamber along the inner surface of the epidermis. They are long, straight hyphæ, with contents densest at the tips. They are composed of greatly elongated cells which branch but sparsely and make few or no haustoria. A runner may strike a host cell squarely, as in Plate 3, A, at *b*, stop, thicken as if about to make a haustorium, and then bend around the obstruction and grow on. These stolons are obviously not self-supporting but derive their food from the central mycelium.

Other hyphae push out towards the surface of the leaf and branch freely just below the epidermis, initiating uredinia. In Plate 3, B, is shown a portion of a young uredinium, *a*, which has not yet broken through the epidermis *b*. It is drawn from material taken seven days after inoculation. The spores and their stalks are binucleate and filled with dense cytoplasm. The uredinium is under considerable pressure from the resistance of the epidermis that it is lifting and the young spores are somewhat bent and flattened by it. Soon after this the epidermis is ruptured and the stalks of the spores, as well as the basal cells below them, elongate. The first spores are freed, their stalks wither, and other spores push up between them.

While the spores and the matted hyphae just below them are richly supplied with food, the intercellular mycelium of the central area is nearly empty and the haustoria are pale and transparent. The host tissues are living and not seriously impoverished.

Details from older material of the central mycelium are drawn in Plate 2, F and G. In F is a portion of a hypha which has contributed much of its substance to the peripheral growth of the fungus. The rich granular cytoplasm of younger hyphae (as seen in Pl. 2, B, D, and E) is reduced here to a few delicate transparent strands. The nuclei persist longer, and this affords an exceptional opportunity to study the structure of vegetative rust nuclei (F, *a* and *b*), for the large nucleolus and chromatin network of the nucleus stand out very clearly in the transparent medium. Later, the nuclei also disappear from these hyphae, leaving only the orange-stained hypha walls.

In Plate 2, G (taken from material 11 days old), the mycelium surrounding the host cell is practically void of stainable material. Three large haustoria extend into the cell. They, too, have lost much of their contents, and in the relatively transparent remainder of each one is to be seen a denser rounded body (*a*, *b*, and *c*), having the size and appearance of a nucleus. The evacuation of the haustorium may be carried so far as to leave it nearly clear and outlined only by the delicate limiting membrane.

In Plate 3, C, is drawn a small portion of a section through a 15-day infection. The host cells are exhausted and their contents partially ag-

gregated. The development of mycelium below the uredinium and through the interior of the leaf is less massive than in Baart. The mycelium from one surface of the leaf to the other is empty. Even the basal cells of the uredinium itself are nearly empty. Many spores have been formed and liberated, as the withering stalks attest. A few spores still remain and show the usual heavy walls with minute warts, and the four equatorially-placed germ pores. Little further growth and spore production would be expected from this part of the uredinium, however.

Effect on Nuclei and Plastids

The results of a study of nuclei and plastids in infected Kanred tissues, similar to that of Baart, are given in Table III. The same methods and magnifications are used.

In comparing these with those of Baart, the first point that comes out clearly is that both nuclei and plastids in the leaves of healthy Kanred seedlings are decidedly smaller than in Baart. The figures for the normal Kanred nucleus are 90 by 70. It is small, dense, and rounded. In Baart the nucleus is 115 by 70. The average healthy Kanred plastid is 45 or 50 by 25, as opposed to 60 by 25 in Baart.[4]

Under the conditions of this experiment, at least, Kanred nuclei expand rapidly at the center of an infection, attaining the maximum size a week after inoculation. The percentage increase in volume appears to be about the same as in Baart. The outlying districts of the infection are affected much sooner here than in Baart. Even at the margin of the mycelium and beyond it, the nuclei are beginning to undergo the same change. The figures seven days after inoculation (Table III, nuclei 7b) 130 by 88 at the center of the infection, 111 by 71 at the margin, and 101 by 71 just beyond the fungus, as opposed to 87 by 71 at the end of the same section, show clearly the rapid radial spread of this effect of the fungus.

By the ninth day, when the uredinia are breaking the epidermis, a few of the centrally located nuclei have collapsed and are flattened or irregular in form. A few of the nuclei live on, even in older material, but the majority at the center and some in outlying regions collapse and stain a dense uniform red in which no trace of nuclear net remains.

[4] Figures in this and the following paragraph are the sums in millimeters of the two diameters of 10 nuclei or plastids × 1130.

TABLE III.—*Comparative size of host nuclei and of plastids in or near infections of Puccinia graminis tritici form III of different ages on seedlings of Kanred wheat* [a]

Days after inoculation.	Center of infection.	Margin of infection.	Adjacent uninfected tissue.	Normal or nearly normal tissue.	Center of uredinium.	Margin of uredinium.	About 20 cells farther out.	End of section.
HOST NUCLEI								
0				90×70				
7a	127×84	101×52		96×65				
7b	130×88	111×71	101×71	87×71				
9a					127×62	114×78	101×67	95×74
9b					127×69	117×77	94×65	88×67
11a					110×73	97×65	113×60	98×62
13					116×65	113×70		110×76[b]
15					115×54	103×71	98×76	112×75
PLASTIDS								
0				45×25				
7a	49×26	50×29	42×26	48×25				
7b	38×22	40×20	34×16	43×20				
9a					37×16	42×20	42×19	43×24
9b					38×24	42×20	44×23	51×28
11					37×21	42×22	36×21	38×24
13					28×22	34×22		37×18
15					30×22	30×19	32×18	35×22

[a] Each figure given represents the sum in millimeters of the diameters of 10 nuclei or plastids × 1130.
[b] Secondary uredinium forming.

In the 13- and 15-day infections recorded (Table III, 13, and 15) there is a secondary increase in the density of the mycelium and in the size of host nuclei some distance away from the main mycelium (normal). Here secondary uredinia are forming.

The plastids undergo a very slow, steady decrease in size from the seventh to the fifteenth day, and the reduction is almost uniform throughout any given infected area. So far as observed, the reduction is never extreme. At the end of 15 days, under the conditions of these experiments, at least, the plastids are still far from minute (30×20). No outer zone has been noted in which the plastids are markedly smaller, and no period of marked reduction in size followed by partial recovery. Perhaps this is correlated with the fact that expansion of the nuclei takes place sooner and is more widespread here than in Baart.

MINDUM AND PUCCINIA GRAMINIS TRITICI FORM III

MACROSCOPIC OBSERVATIONS

Mindum inoculated with *Puccinia graminis tritici* form III gives an o-type of infection. No spores are formed. Small spots or "flecks" of discolored host tissues occur, but they are surprisingly late in appearing, sometimes not showing until the eighth day after inoculation. Moreover, the flecks are few in number even when an abundance of spores is applied to the leaf, and they differ markedly in appearance from the commoner types of rust flecks. The fleck here consists of a minute circular grayish-white area, uniform in color, and sometimes visible on only one side of the leaf.

MICROSCOPIC OBSERVATIONS

The study of prepared slides shows that the spores germinate readily when placed on the leaves, and appressoria are to be found over the stomata on the day following inoculation. By the end of two days many of the fungi have passed through the stomatal slit and begun the attack on the host tissues.

Entrance Phenomena

As in every combination of rust and host studied so far, some of the fungi do not enter. These usually are in the minority, but always are found. Many of the entries occur on the second day, when both the appressorium and the guard cells with which it lies in contact look normal. On the third day after inoculation the guard cell wall in contact with the appressorium loses stainability (Pl. 4, A), and the central part of the inner wall of the cell is also pale. Slight, if any, changes in the guard cell contents are to be noted at this time. The formation of the central mass of nuclear material at *a* may be a reaction to a stimulus from the fungus, for host nuclei tend to move toward the fungus. There was some evidence of this in Kanred, but it is much more noticeable here. If, as sometimes happens, the appressorium lies at one end of the stoma, the elongated nucleus of the guard cell often will contract into a single lump, lying just under the fungus.

The reaction progresses more rapidly and is more extreme than in other cases studied. The entire wall loses power to stain, and later the cell contents die and become dissolved, first in the part of the cell nearest the fungus and later in more remote parts. The effect may extend

beyond the stoma to adjoining epidermal cells. In Plate 4, B, is shown an unusual case in which a mesophyll cell happens to be in direct contact with the guard cell at *a*, and it, too, shows disordered and partly dissolved contents.

Plate 4, B (taken from material fixed seven days after inoculation), illustrates one or two other points. The guard cell walls are weakened, as is evidenced by the fact that one at *b* was badly broken and torn in sectioning. This is of common occurrence at this stage. Although the wall is weakened, it is not appreciably swollen. This is in contrast with the adjoining walls of the epidermal cells, as at *c*, which have swollen enormously, enabling one to see clearly the layers of which they are composed. The appressorium *d* shows signs of degeneration. It is shrunken and its contents are somewhat disorganized, the nuclei being scarcely distinguishable.

There is nothing unusual about the manner of entry of the fungus. It pushes a thin wedge-like projection through the stomatal aperture and the protoplasm flows through, usually forming the substomatal vesicle just inside the stoma. From the vesicle, the infecting hypha grows out over the end of the guard cell and skims the inner surface of the epidermis until the tip strikes a mesophyll cell. In exceptional cases the infecting hypha develops directly without the formation of the substomatal vesicle. Sometimes two infecting hyphæ are found growing in opposite directions, or a single hypha may branch into several.

Development of the Fungus After Entrance

A general idea of the early relations between fungus and host may be gained from Plate 3, D, a low-power drawing of part of a longitudinal section through the leaf four days after inoculation. In contact with the outer side of the guard cell is the empty appressorium *d*, and on its inner surface is the substomatal vesicle of the fungus, also empty. Two infecting hyphae have formed. The first, at *e*, has already attacked the host cell *f*, with the result that both it and its host are dead. The second, at *g*, is just beginning to form a haustorium in the mesophyll cell, and a branch hypha is growing off toward deeper-lying tissues. Cells at *h* and *i*, at some distance from the dead cell *f*, are plasmolyzed.

Material was available for a detailed study of this first attempt of the fungus to establish relations with its host. As soon as the tip of the infecting hypha meets the mesophyll cell, changes set in preparatory to the formation of a haustorium. Its pair of nuclei divide, one daughter pair of nuclei moves out into the tip of the hypha and a septum forms back of them, separating a short terminal cell, the haustorium mother cell. The nuclei of this cell decrease rapidly in size.

The haustorium mother cell usually is wedged into the angle between a mesophyll cell and an epidermal cell (pl. 4, C), and the haustorium may be formed in either of the two host cells. The history of the fungus may vary somewhat, according to the course taken at this point, for the haustorium usually is killed promptly if it forms in the mesophyll cell, but it may live and function for several days if formed in the epidermal cell.

In the majority of cases the first haustorium forms in the mesophyll cell. An early stage in this procedure is represented in Plate 4, C, which was drawn (× 1460) from material taken two days after inoculation. The infecting hypha ran obliquely to the plane of the section, the proximal

part of the hypha being in one section, C_1, and the distal part (separated by a space in the drawing) in the next section, C_2. The haustorium mother cell, with its pair of minute nuclei, is at *a*, and the young haustorium forming from it at *b*. There is no marked disturbance of the host cell contents, but the plastids and nucleus of the host cell are beginning to collect around the haustorium.

This is the beginning of a violent reaction in the host cell. A slightly later stage, also taken from two-day material, is shown in Plate 4, D. There has been a rapid flow of the host cell contents toward the haustorium at *a*. Two lobes of the host cell at *c* and *d* have been completely evacuated and their walls have collapsed. Cytoplasm, plastids, and nucleus *b* are massed around the haustorium. The cytoplasm and plastids seem to be still alive, but the chromatin network of the nucleus is nearly dissolved. The fungus is less violently affected, although the haustorium mother cell at *e* has collapsed and is dying. In this case, the fungus has strength for a second attack, as there is a fresh young hypha at *f*.

A more advanced condition (still taken from two-day material) is represented in Plate 5, A. The infecting hypha and haustorium mother cell at the end of the guard cell at *a* are shriveled and dead. The attacked mesophyll cell also is dead. It is stained a deep red at the end near the fungus, and the color fades toward the other end. Evidently here, too, there was a concentration of living matter about the fungus. The haustorium *b* lies within a narrow clear zone, making it a conspicuous object within the cell. This clear space was present, but less sharply defined, in the earlier stages. The host nucleus *c*, which is pressed close against the haustorium, is dead and has lost all trace of inner structure.

The damage in this case does not stop with the cell directly attacked. At *d*, where the dead cell touches another cell, we find the living cell slightly plasmolyzed, its nearest cytoplasm altered in appearance and the contact wall between the two cells slightly swollen. At *e*, a contact with a third cell at a greater distance from the fungus, the damage is negligible. One or two plastids have disintegrated, but there are no other visible changes.

When the first haustorium is formed in a mesophyll cell, it usually results in the massing of a large part of the cell contents about the haustorium, followed by the immediate death of both the cell and the haustorium. This is a severe check on the fungus, as its limited resources are seriously depleted. When the fungus possesses vigor enough for a second attack, it goes on. In looking through the older material, however, it is not unusual to find minute infections consisting of an empty appressorium and substomatal vesicle, dead colorless guard cells, and a single dead mesophyll cell. This shows that the fungus may die after making a single haustorium. More commonly, however, several host cells are attacked in succession before the fungus is exhausted. For a time these later attacks result like the first.

Still later, however, the host reacts less violently to the fungus. The milder reaction may be due to enfeeblement of the fungus; or to some response in the adjacent host tissues to the presence of the fungus; or, perhaps, to the fact that the fungi capable of evoking the most violent reaction in the host are already killed by it; or, conceivably, even to a slightly varying resistance to the rust in different parts of the same leaf. The latter is least likely, as it would be difficult to explain why it is invariably the first cells attacked that respond most violently.

At any rate, in material of Mindum fixed seven days or more after inoculation with this rust, haustoria are to be found which have not caused a complete collapse of the host cell. The nucleus and a portion of the cytoplasm move to the haustorium, but the remainder of the cell contents keeps its place, and the cell as a whole retains its original shape. There are no empty lobes of the cell with walls collapsed.

A typical example is represented in Plate 5, B. At *b* is a group of hyphae with scant contents, and at *a* is a haustorium connected by a slender neck to its mother cell outside. The nucleus *f* near by is dead. Enveloping both the body and neck of the haustorium is a thick irregular sheath, greater in bulk than the haustorium it covers. Both the haustorium and its sheath are dead. When newly killed, it makes a most conspicuous object in the cell, as it stains intensely. Later on it loses power to stain and becomes glistening and transparent. The damage done by the fungus in this case has passed beyond the limits of the cell it entered, for there is plasmolysis in the next cell at *c*, a swollen wall at *d*, and a massing of nucleus and cytoplasm in an adjoining cell at *e*.

Dozens of haustoria of this same general appearance are to be met with in older infected material. The body of the haustorium usually remains small and dense, often spherical, as if unable to expand freely in its heavy sheath, and it soon dies.

An attempt was made to study the nature and origin of this haustorial sheath. In Plate 5, C, is a haustorium, *a*, and condensed around it in more or less concentric layers are materials continuous at their periphery with the cytoplasm of the host cell. The nucleus *b*, in attendance as usual, is collapsed and dead. Another case was seen in which host cytoplasm was banked around the neck and the basal half of the body of the haustorium. This haustorium had expanded almost normally and its contents opened into a reticulum. In Plate 6, A, at *f*, is another haustorium, about which the host cytoplasm is concentrating. It is interesting, too, in this connection that in Plate 5, B, at *e*, a cell not attacked directly by the fungus, the nucleus, plastids, and cytoplasm have banked up on the side of the cell nearest to the cell attacked by the fungus. While the evidence is not conclusive, it seems probable that the sheath originates from host cytoplasm, although the possibility is not excluded that the haustorium itself contributes to it by secretions of some sort, or that host, or fungus, or both, secrete more or less of cell wall substances around the haustorium.

For some reason the presence of the haustorium seems to induce host cytoplasm to move toward it and bank around it. Each seems to be toxic to the other; at least both die very soon. The host nucleus almost invariably is found alongside of the haustorium, and it, too, dies quickly. The end of this struggle appears to be the digestion of both the haustorium and its covering and often the death of the cell containing them. In Plate 5, D, at *a*, fixed 11 days after inoculation, haustorium and sheath are transparent, having lost all affinity for stain, and only by reducing the light could the limits of the former haustorium within be discerned. The dead nucleus *b* was the only stainable object left in the cell. The mycelium near by at *d* also is dead and transparent.

Haustoria in mesophyll tissues fare ill, but in epidermal cells a haustorium may attain to full size before causing any perceptible disturbance. Plate 5, E, shows a full-sized healthy looking haustorium, *a*, in an epidermal cell. It has retained its normal connection with the empty hausto-

rium mother cell at *b*, and seems to be functioning. There is a rich supply of cytoplasm about its base and at *c*, but little more than would be there if the fungus were in a congenial host. In other parts of this same infection, however, the mesophyll cells attacked are dead or dying.

As was mentioned earlier, the first haustorium made by the young fungus may be formed in either the epidermal cell or the mesophyll cell with which the haustorium mother cell lies in contact. When, as happens occasionally, it forms in the epidermal cell, it may attain to full size and function for several days. This is a decided advantage to the fungus at this critical stage in its life, as it obtains nourishment enabling it to form a considerable mycelium. Plate 6, A, illustrates this. The section is cut obliquely, showing the two guard cells, *a*, in perspective, with the empty appressorium *b* fitted into the hollow between them. At *c* is an oblique section through the accessory cell of the stoma. The first haustorium in the epidermal cell at *d* was bisected in sectioning, the other half of it being found in the next section. This material was fixed seven days after inoculation, so this haustorium is several days old, but it is still living and has caused very little disturbance in the host cell. Perhaps because of this aid the fungus has formed a fairly rich mycelium which extends through several sections. Nearly two dozen mesophyll cells have been entered by haustoria, and all are dead or dying. Two of these, at *e* and *f*, are included in the drawing. In *f* the engulfed haustorium forms a dark-stained body, and a large part of the contents of the cell is contracting around it. A few sections farther on there is a second haustorium in an epidermal cell (Pl. 6, B, *a*). This and the first haustorium are the only living haustoria in that entire mycelium.

It is not obvious at first sight why haustoria of the same individual should thrive longer in epidermal cells than in the mesophyll cells adjoining them. Epidermal cells have no plastids, and perhaps they differ chemically in other respects from mesophyll tissue, and either do not possess the power to produce the substance that kills haustoria or possess it in lesser degree. Or, it may be that a haustorium escapes longer in an epidermal cell for the simple reason that the latter can not readily mobilize its forces. The epidermal cell is very large and possesses the minimum of living material spread out as a thin layer lining the long walls. It would take time for the living matter of such a cell to flow to the point of invasion and surround the haustorium.

An interesting mesophyll cell was seen in which a haustorium formed and grew to a considerable size but was finally killed. A later haustorium formed in the same cell and met almost no opposition. It looked normal and was covered by only a thin layer of host cytoplasm. The cell perhaps was too nearly exhausted by the first attack to resist a second one effectively.

In course of time, however, even an epidermal cell can kill a haustorium. There are signs of this in one already mentioned (Pl. 6, B), for the haustorium *a*, though still living, is covered by a fairly thick orange-stained layer and the nucleus *b* of the epidermal cell is near at hand. In older material (Pl. 5, D, fixed 11 days after inoculation) the haustorium in the epidermal cell at *c* is incased in a thick layer which is colorless and faintly stratified. The haustorium (red-stained) is dead and also shows irregular stratification.

Only a few of the hyphæ in infections a week or more old show stainable contents, and these chiefly at the growing tips. Even the latter are relatively scant in content and starved in appearance. The fungus can

extract but little food from its host, and each attempt to form a haustorium wastes some of its substance, so it soon exhausts itself.

The number of host cells killed by being entered directly by the fungus ranges from one to about twenty or more, a common number being five or six. The damage done by the fungus is not limited to this primary effect, however. Substances from this "primary area," if one may so designate it, diffuse into neighboring tissues, affecting them to a lesser degree. Probably without this secondary damage the fungus would produce few, if any, flecks, for the patch of dead cells in the primary area seldom is large enough to be seen with the naked eye.

Whether the substances that diffuse outward from invaded tissue are the same ones that are excreted by the haustorium into the cell, or others formed there as a result of their presence, or both, is uncertain.

It is possible that the fungus does harm in another way. Guard cells of stomata occupied by the fungus usually die, although, so far as known, the appressorium does not enter those cells to form haustoria. This at once suggests the possibility that in later growth portions of living mycelium in mere contact with the outer surface of mesophyll cells could secrete substances that would penetrate the cells and affect their contents. Such an effect would be slow and its existence would be hard to prove. It would be difficult to find a case where the harm was due unquestionably to this one factor, for a hypha growing out from the center of the infection is rarely much in advance of the damage done by the diffusion outward of substances from the dead host cells of the primary area. It is possible, however, that it does occur and is a minor factor in the situation.

These secondary effects of the fungus are not as violent as the primary ones, and the tissue so affected differs markedly in appearance.

One of the secondary effects of the fungus is plasmolysis. Even as early as on the fourth day after inoculation (see Pl. 3, D) several cells, *h* and *i*, at some little distance from the fungus, show the cell contents shrinking away from the cell wall and drawing together into a ball. Very soon after this a layer of tissue, which may be three or four cells thick in all directions from the fungus itself, shows decided plasmolysis. If this effect reaches the long straight cells of the bundle sheath, which seem to serve to some extent for conduction of food materials in the leaf, the plasmolyzing agent follows them rapidly for a much greater distance, sometimes from one end of the section to the other. At first the plastids of the plasmolyzed cells look normal. Later they decrease in size. This may be due partly to starvation, as the shrunken cell contents have lost normal relations to other cells. Still later there is a partial or complete recovery from plasmolysis in the outer part of the affected zone, which now betrays its former trouble only by the minuteness of its plastids and the abnormal condition of a few of its nuclei. The tissue nearer the source of trouble also may recover if not too severely affected, but more commonly cells close to the primary area are quite empty and perfectly clear, although retaining their original shape.

Another secondary effect of the fungus is a swelling of the host cell walls. This occurs irregularly, here and there, never affecting all of the cells within the influence of any one fungus, nor even all of the wall of any one cell. Moreover, one infection differs markedly from another in this respect.

The guard cell walls are rarely swollen, although they are usually altered chemically. That these walls can be affected, however, is shown in Plate 6, C, where the cell lumen actually is pinched in two by the coming together of the opposite walls of the cell. It is noteworthy, too that although the walls are strongly affected, a part of the cell contents still is living.

Two fungi entered adjoining stomata on the same leaf and grew in divergent directions. The two were of about the same age and size and the cells killed directly by the fungus were similar in appearance. A close comparison of the two revealed a notable difference in the matter of swollen walls.

A portion of one of those two fungi is drawn in Plate 6, A, already referred to. A slightly swollen wall is found at *g* and nowhere within the influence of this fungus is there any more pronounced effect. In the other of the two fungi, on the contrary, there are several cells with markedly swollen walls (Pl. 6, D). The lamination of such a wall can be made out clearly, and at *a* some of the wall material, perhaps the middle lamella, seems to be almost liquefied.

These differences between infections have been noted repeatedly. It may be that there are two strains of the fungus here, differing only in their effect on the cell walls. The culture from which this material was inoculated was not started from a single spore, so this would be possible. Both give the o type of infection. The difference is one of degree, not of kind. Another less likely explanation is that the composition of host cell walls may differ slightly in different parts of the leaf.

One odd fact is that host cells whose contents react violently to the fungus do not show swollen walls, and the swelling is often greatest some distance away from the fungus. In Plate 6, E, (four days after inoculation) the cell at *a* was entered by the fungus and killed and has completely collapsed. So far as can be judged its wall is still thin. In the near-by cells at *b* and *c*, on the contrary, the walls are swelling rapidly and have arched outward, severing the contact-wall between them. The granular matter between the two swollen walls is probably the disintegrating remnant of the middle lamella. So, too, in Plate 5, B, the wall is most swollen at *d*, a little distance away from the fungus.

The finest specimens of swollen walls are found in much older material. One of these was drawn at high magnification (×1460) in Plate 7, A, from a leaf fixed 11 days after inoculation. These swollen walls take no stain (with the triple stain at least) and are transparent, but by reducing the light every detail stands out clearly. Mesophyll tissue has fairly thin walls, and it seems almost incredible that a corner where two or three cell walls meet, such as the one at *c*, could swell into the bulky masses seen at *a*. Yet, such is obviously the case, as each part of this enormous wall can be traced back directly into ordinary unchanged walls. The distance from *a* to *b* is the actual thickness of the wall, and every detail of its structure is proportionately enlarged. Such a wall must be almost liquefied. It is interesting to note that even here an occasional nucleus and a few plastids have survived. Nothing corresponding to this has been observed in infections of this rust on Baart or Kanred.

The death of the fungus may occur a day or two after its entry through the stoma, or it may be deferred for a considerable period. The great majority are dead before the ninth day. It is a surprising fact, however, that the few that survive this long may continue for a much longer period. It is not known how long, but material fixed 15 days after inoculation still shows an occasional infection with a few feeble living hyphae. The fungus carries on a meager existence, barely living from day to day, and never rising into vigor enough for any attempt at spore formation.

The appearance of the tissues in one of the small flecks caused by the fungus is represented in Plate 7, B. It is a longitudinal section of the leaf 15 days after inoculation. Below the stoma, at the point of entry, *d*, are several empty hyphae, and scattered threads occur elsewhere among the cells. One or two of them still contain a little cytoplasm. Some of the earliest cells attacked (*f*, *g*, and *h*) have shrunken and their contents are so transformed as to appear a homogeneous blur. The cells attacked later have retained their shape, except where distorted by a swollen cell wall. Several cells contain rounded haustoria, with thick stratified coverings (*a*, *b*, *c*, and *e*). Sometimes the haustorium is the only object left in such a cell, the nucleus and plastids having disappeared. Some of the near-by cells are also empty, while others contain a nucleus and minute plastids. This dead area is large enough to be seen as a minute white speck in the living leaf.

Effect on Nuclei and Plastids

A study of nuclei and plastids in infected areas in Mindum, corresponding to the studies of Baart and Kanred, is summarized in Table IV.

In studying these records, several points should be borne in mind. Mycelia vary widely in length of life and in size. Some were dead before the fourth day and others maintained a few living hyphae to the fifteenth day. Some mycelia remained minute and their effect upon the host extended but a short distance, while others spread farther and their influence extended through a broad zone beyond the fungus itself. Besides this, healthy host nuclei vary in size more than in the other hosts, ranging from 85 by 64 to 108 by 77, and this makes it more difficult to estimate accurately the effect of the fungus.

In spite of these irregularities, however, several points are shown fairly well. Extreme expansions of host nuclei have not been found in infected areas of any age. Nuclei in the first cells attacked by the young fungus are destroyed rapidly and become indistinguishable. In older infections the nucleus of a cell containing a haustorium also collapses soon and dies, but may be seen for a time as a flat, red-stained disk.

In a narrow zone of tissue distant only one or two cells from the fungus the nuclei are uniformly collapsed and dead and lie flattened against the cell wall. An occasional cell farther out is in the same condition. This collapse takes place remarkably early, being quite as marked on the fourth day as in older material, the short diameter of these nuclei (Table IV, first column) being between 40 and 50 throughout the series. The dimensions give little evidence of expansion previous to the collapse, for the long diameter of these flattened nuclei is (with one exception) but little over 100.

TABLE IV.—*Comparative size of host nuclei and of plastids in infections of Puccinia graminis tritici form III of different ages on seedlings of Mindum durum wheat.*[a]

Days after inoculation.	2 cells beyond fungus.	3 or 4 cells beyond fungus.	8 to 10 cells beyond fungus.	Near end of section.
HOST NUCLEI				
4	100×43	.....	110×77	108×77
7a	118×42	115×78	110×71	97×72
7b	105×42	118×55	109×60	100×68
9	103×40	112×80	113×77	96×72
11	81×43	112×52	95×53	90×62
15	101×49	97×60	88×59	85×64
PLASTIDS				
0	.....	.....	.....	50–60×30
4	52×31	.....	50×24	61×32
7a	32×15	37×26	41×19	52×26
7b	31×13	38×20	36×22	46×23
9	32×18	34×18	36×19	52×28
11	13× 8	26×18	55×32	57×26
15	15×12	24×13	30×16	41×22

[a] Each figure given represents the sum in millimeter 1of the diameters of 10 nuclei or 10 plastids×1130.

In the tissue three or four cells away from the fungus, and to a lesser extent even farther out, the nuclei are usually slightly enlarged (cf. second, third, and fourth columns in Table IV) and contain an open network.

Plastid size in these same areas also is given in Table IV. The normal plastid is 50 to 60 by 30, varying somewhat in different tissues. In the cells containing haustoria, the plastids disappear. In the narrow zone of tissue distant only one or two cells from the fungus, where we find the nucleus collapsed and dead, the plastids are nearly normal in size (52 by 31) on the fourth day, but undergo a slow and steady decrease from then on. The figures for these plastids on the fifteenth day (15 by 12) represent the plastids found, but do not represent the whole situation, as many of the cells are by this time quite empty. At all ages the smallest plastids are in the cells nearest the fungus, and the damage grows less as the distance from the fungus increases.

Under these conditions, then, the nuclei near the fungus die, and those a little farther off undergo but slight expansion. The plastids are smallest nearest the fungus, and show progressive decrease with age.

COMPARISONS OF FORMS III AND XIX ON BAART, KANRED, AND MINDUM

Several questions arose in connection with the study of the death of guard cells of stomata occupied by appressoria of the rust. How marked is the difference in intensity and rapidity of this reaction on different hosts? What connection, if any, exists between this reaction and the entry of the fungus, or its exclusion from the host? Is there any correlation between the strength of this reaction and congeniality, or lack of it, between the rust and its host? Would two specialized forms of the rust differ in the intensity of this reaction on any given host, and would such a difference be correlated with resistance or susceptibility of that host to the rusts?

In Table V the data for forms III and XIX on Baart, Kanred, and Mindum are summarized. The two rusts differ markedly in their ability to attack these hosts. Baart is susceptible to both, Kanred is susceptible to form III and immune from form XIX, while in Mindum these relations are just reversed, Mindum being immune from form III and susceptible to form XIX. Stomata occupied by the fungi are divided into four classes: Those normal or not visibly affected, those with the central part of the guard cells more or less injured, those in which the guard cells are dead, and those in which not only the guard cells but ends of adjoining epidermal cells are affected. The time of year in which the material was grown, the age of the fungus (number of days after inoculating the plants), the total number of stomata counted, the percentage of the fungi that entered the host, and the percentage belonging to each of the four classes for each lot of material are shown in the table. Each lot represents a given age of the fungus, and the different lots, fixed from two to eight days after placing the spores on the leaf, show the progress of the reaction.

TABLE V.—*Data comparing specialized forms III and XIX on Baart and Kanred common wheats and Mindum durum wheat, with reference to effecting entry and causing injury*

Host variety.	Type of infection.	Month when grown.	Days after inoculation.	Fungi.		Condition of guard cells of stomata.			
				Number counted.	Percentage entering.	Not injured.	Partly killed.	Dead.	Dead and adjoining cells injured.
FORM III.									
						Per ct.	*Per ct.*	*Per ct.*	*Per ct.*
Baart	4	October	6 & 7	68	30	53	41	6	0
Kanred	3	April	2	58	5	100	0	0	0
		do	4	99	17	23	41	36	0
		do	6	166	10	6	33	51	10
Mindum	0	July	4	60	72	8	58	34	0
		do	7	100	77	0	6	38	56
FORM XIX.									
Baart		December	4	24	75	100	0	0	0
	4	do	8	60	30	92	8	0	0
Kanred	0	April	2	209	11	100	0	0	0
		do	4	200	18	21	37	42	0
		do	6	108	14	0	29	55	16
Mindum	3	do	4	50	78	18	26	44	12
		July	6	41	30	12	49	32	7
		March	7	39	23	2	14	46	38

A study of Table V shows that there is but little difference between forms III and XIX in their effect upon the stomata. On the whole, form III produces a slightly stronger reaction. This is particularly noticeable in Baart, where only 8 per cent of the stomata show any trace of the reaction with form XIX even after eight days, and nearly half of the stomata are affected with form III after six or seven days. The difference between the two rusts on the stomata of the other hosts is scarcely perceptible, although the two rusts differ markedly in their ability to grow upon these hosts. The effect of these two rusts upon the stomata bears no obvious relation to immunity or susceptibility and would appear to be independent of it.

On comparing the effect upon the three hosts, however, marked differences are seen. With both forms the effect on the stomata is least in Baart, intermediate in Kanred, and greatest in Mindum. So far as the microscope reveals, the effect is the same in kind on the three hosts, but differs in degree. This difference can be attributed only to differences in the hosts.

It seems fairly certain that the appressoria secrete some substance which penetrates the guard cells. It is natural to suppose that this may affect the entry of the fungus, but no such relationship is obvious from the data at hand. The effect on the stomata is least in Baart with form XIX and greatest in Mindum with form III; yet the percentage of entries in the two cases is about the same. Of form XIX, 75 per cent have entered Baart four days after inoculation, although in that material there were no visible effects upon the guard cells. Of form III on Mindum, 72 per cent have entered at the end of four days, and in that material only 8 per cent of the guard cells are unharmed, 58 per cent are partly killed, and 34 per cent are dead.

The percentage of entries in the various cases is decidedly uneven. Take, for example, the two lots of Baart inoculated with form XIX. The two sets of seedlings were grown side by side in the greenhouse in December, 1920, given parallel treatment, and both were inoculated on a dark, rainy day; yet, for some reason, one showed 75 per cent of entries and the other, 30. Moreover, as already pointed out, different parts of the same leaf may vary in this respect. The percentage of entries in Kanred also varies, but within much narrower limits, and the entries are always markedly fewer than on either of the other hosts.

DISCUSSION

It has been shown that changes occur in the guard cells of stomata occupied by appressoria. The wall just below the appressorium is first affected, then the adjoining cell contents, then the inner walls of the guard cells just below the appressorium, then the ends of the guard cells, and sometimes even the nearer parts of cells beyond. This process suggests that the fungus is secreting some substance or substances, perhaps enzymatic in character, which diffuse into the host tissue, even spreading into neighboring cells.

It is possible that only one substance is secreted and that it first softens the walls and then kills the cell contents. Brown (9) has shown that a single enzym secreted by the germinating spores of *Botrytis* dissolves the cellulose walls of its host and then kills the cell contents. It has no action, however, on the cuticle. Only when the germ tube has ruptured the cuticle by mechanical pressure is the enzym able to produce results.

The varying intensity of the reaction on the different hosts (Table V) when tested with a given form of the rust can be due only to inherent differences in the hosts. Brown (*9*) found that the enzym of Botrytis (pectinase) referred to above attacked the cell walls of various higher plants, but was quite unable to affect the walls of the mosses and hepatics tested. This was ascribed to differences in the composition of the cell walls. It may be that lesser chemical differences in wall materials, such as might occur in nearly related varieties of plants, could still be great enough to cause differences in the degree of the reaction when exposed to the enzym.

Smith (*34*) observed an alteration of the host cell walls caused by Erysipheae that was very similar to the one found in rust attacks:

> The cell wall around the point of penetration (of the epidermis) is more or less altered and dissolved. Seen from the outer surface of Poa (*Erysiphe communis*) and Eupatorium (*Erysiphe cichoracearum*), there is an area surrounding the point of penetration which is entirely colorless, clear, and shining. The remaining portions of the epidermal wall stain with safranin.

In both of the cases cited, as well as in those of *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scribn., studied by Dey (*10*), and *Sclerotinia libertiana* Fuckel, Boyle (*8*), and many others, the fungus enters through the epidermal wall, and this reaction assists its entrance by softening or dissolving the cellulose layers below the cuticle. In rusts, on the contrary the entrance is through the stoma.

In this connection it should be recalled that, although the germinating urediniospore and aeciospore of rusts enter only through stomata, the sporidium of many rusts, including *Puccinia graminis* Pers., penetrates the epidermal wall directly. De Bary says (*4, p. 26*):

> Letztere (i. e. sporidia of *Puccinia graminis*) hatten in Masse gekeimt und überall sah man eine Menge von Keimschläuchen die Wand der Epidermiszellen *durchboren* und ins Innere dieser eindringen.

In 1921, Waterhouse (*45*) also studied the entrance of the sporidium of *Puccinia graminis tritici* Erikss. and Henn. The sporidium produces a short germ tube which becomes attached to the epidermis and then pushes a very fine proboscis-like tube through the cell wall.

> No alteration in the nuclei when these were present in the epidermal cells or in the cellulose layers underlying the cuticle could in any case be detected at this stage.

The sporidium apparently pierces the epidermal cell wall in much the same fashion that the haustorium mother cell pierces a mesophyll cell wall in making a haustorium. No evidence is given of any alteration of cell walls due to secretions, although such secretions would assist the entrance of the fungus here. This makes it more surprising to find such secretions at another part of its life cycle, where the natural openings of the plant are utilized for entrance.

The utility of this alteration of guard cells, so far as the entrance of the fungus is concerned, is open to question. As already noted, the percentage of entries may be quite as great where the reaction is least as where it is greatest. Of course, the possibility is not excluded that the first stages of this reaction may affect the entry, although one can not tell from the present data whether the result would be to help or to hinder. It is conceivable that the first effect might be a stimulation of the guard cells which would result in their opening. The first effect, however, might be a loss of elasticity of the guard-cell walls that would result in their inability to arch out, in which case the stoma would re-

main closed; or a loss of turgor in the cell contents that also would prevent the opening of the stoma. Later on, the guard cell walls might be so softened that the fungus could push through without the normal opening of the stoma. The last seems least likely, as entries usually take place before the stomata are so far deteriorated. The majority of the fungi enter, except in Kanred, and the guard cells die in much the same way when the fungus does enter as when it does not. Perhaps the entry of the fungus depends on its promptness. It may be that the fungus must enter before it destroys power of motion in the guard cells.

Loftfield (*22*), in his studies on the behavior of stomata, found that stomata of cereals are closed at night, open partly (only 30 per cent of the maximum aperture) in the morning, close before noon, and remain closed until the next morning. Stomata of wheat plants grown in the greenhouse open wider and remain open longer than those of plants grown out of doors. He notes that cool and rather humid weather and less sunshine favor the opening of wheat stomata, and points out the relation of this to the spread of wheat rust. Cereal stomata rarely show the maximum opening, and many are closed even during the morning. He says (*22, p. 40*):

> It is not definitely known whether a few stomata with more accessible water supply do the opening on days of unfavorable conditions, or whether groups of stomata open and shut very rapidly and at different times. Direct observations on the same leaf would indicate the former, but the fact that open and closed stomata occur in groups, and that the stomata of cereals can open and close with amazing rapidity, makes the latter hypothesis possible.

And (*p. 45*)—

> In all cereals the tendency seems to be to operate with many closed stomata at all times.

This tendency in wheat to keep many of its stomata closed may explain the fact that some apparently vigorous appressoria remain outside. If the stoma remains closed for two or three days, the secretion by the appressorium probably would render the mechanism of the stoma inoperative. It would lose power to open and the fungus would be excluded. The fact that open and closed stomata are found in groups may explain the differences in the percentage of entries in different parts of the same leaf. In Kanred, with its smaller stomata, these peculiarities in stomatal behavior might result in the exclusion of a much larger percentage of the fungi than in varieties with large stomata.

In Mindum, the host in which the effect of the fungus on the stoma is most pronounced, portions of the mesophyll cell walls in infected areas undergo marked swelling. No such result was seen when this rust grew on the other hosts. The difference, whatever it be, lies in Mindum.

As noted before, these swellings do not occur, so far as can be discerned, in the walls of host cells whose death is most violent. In other words, where the substances secreted by the fungus are most concentrated, and the host cells are killed rapidly, the swelling of walls does not occur, or at least it can not be recognized. Perhaps other substances formed during the death of the host cell inhibit the reaction. The swollen walls are seen best farther out where the host cells are still living, although somewhat harmed by the diluted secretions that have diffused out to them, or in older infections where the reaction is milder. It is not clear whether this means simply that the substance secreted by the fungus is free to act there, or that the diluted secretion is best suited to the purpose, or that the active participation of a living host protoplast is necessary to produce the swelling.

The drainage of the protoplasm from the central mycelium to its peripheral hyphae when spore formation begins has been noted by Pole Evans (*12*) in leaf rust of rye and in other rusts. It may be of general occurrence. It is not easy to learn how this transfer of material takes place. Of course, it is conceivable that this process involves the actual flowing of living protoplasm along the hyphae and through the septa. Seifriz (*33, p. 281*), in microdissection of Rhizopus, has shown that its protoplasm, particularly in the outer layer, may have high viscosity and in occasional filaments the protoplasm is a "firm jelly."

> By pressure with a needle some distance behind the torn end (of a hypha) the rod of protoplasm can be made to ooze out like oil paint from an artist's tube. This protoplasmic jelly is sufficiently rigid to hold its shape until dissected.

Of course, this may be no evidence of the consistency of fungous protoplasm in general, but it is at least suggestive. It would be difficult to understand how viscous materials could pass through the septa intervening on the way to the growing tips. Perhaps the contents of haustoria and hyphae are reduced by some autolytic or digestive process to simpler soluble forms that would be more readily transportable.

In Mindum only the growing tips of hyphae in older infections show stainable contents. All the older hyphae look empty. The first assumption is that the host in some way is poisoning the hyphae. It certainly destroys the haustoria, but there is little evidence of positive harm done outside of the host cell. In the first attacks of young fungi, where the host cell reacts violently, the haustorium mother cell outside may collapse. In older infections no visible evidence of injury has been detected. It may and probably does occur to some extent, but there is no such proof of it, as was seen, for instance, when form XIX was grown on Kanred (*2*). Many of the hyphae of infections in Mindum are empty, but so were those in the central mycelium of this rust on Baart at the same age. Perhaps here, too, and by the same process, the contents of the hyphae are continually transferred to the growing tips, leaving the older hyphae empty.

It was once believed that the power of a rust to enter a plant was an index of its power to infect that plant. Miss Gibson (*15*) showed that germinating urediniospores enter plants quite unrelated to their natural hosts, but do not form haustoria. She concludes that—

> it is the power of the hyphae to form haustoria which we must take as an index of infective capacity.

This conception, too, has been modified. In both Mindum attacked by form III and Kanred attacked by form XIX haustoria are begun but are destroyed by the host, and the fungus fails to become established.

When form III produces a haustorium in a cell of Mindum, the nucleus and part or all of the cytoplasm of the cell flow toward the haustorium and surround it. It is not fully proved, however, that the heavy sheath about the haustorium of older infections is made of condensed disintegrating cytoplasm.

Other types of sheaths about haustoria have been described. Harper (*17, p. 664*), in studies of Erysipheæ, says:

> Innerhalb der Zelle schwillt es (the haustorium) zu einer langliche Blase an, die sich fest an den Kern der Wirthszelle anlegt, um endlich von letzterem vollständig umschlossen zu werden.

This nucleus becomes disorganized and—

> bildet dann nur eine dicke körnige Schicht um das Haustorium.

In Mindum, also, the host nucleus moves to the haustorium but does not become its sheath, for the nucleus can be seen alongside of the haustorium long after the sheath is fully formed.

Smith (*34*), also studying Erysipheæ, concludes that—

> the host nuclei and the haustoria are indifferent to each other.

He notes that as the haustorium penetrates the outer wall of the epidermal cell the host secretes cellulose, forming a layer surrounding the growing haustorium. The heavy sheath of the haustorium in that case consists of cellulose secreted by the cytoplasm of the host cell and partially disintegrated by secretions of the fungus. Of course it is not impossible that some cellulose is secreted about the haustorium in Mindum, but it is doubtful whether the sheath as a whole could have such an origin, for cytoplasm about the haustorium is killed quickly. It is possible that an originally thin cellulose sheath later becomes partially disintregrated and enormously swollen, like the outer walls of some of these cells.

The motion of living protoplasm toward the haustorium, as it occurs in Mindum, is by no means unique. Eriksson (*11*), in his studies of *Puccinia graminis* Pers. on oats, figures the nucleus and haustorium uniformly in contact with each other (pt. IV, Pl. 2). This contiguity plays a part in his theory of "Mycoplasm." Rosen (*32*), in his study of *Puccinia asarina* Kunze on Asarum, noted that the haustorium is close to the host nucleus and often wrapped around it. He interpreted this to mean that the haustorium grows to the nucleus rather than the reverse. Blackman and Welsford (*7*), in studies of infection by *Botrytis cinerea* Pers., state:

> As the hyphae penetrate through the epidermis, the cells of the palisade parenchyma become affected. First the nuclei move upwards towards the epidermis, then gradually they begin to disintegrate . . .

They did not feel certain, however, that this nuclear movement was a response to the fungus. Dey (*10, p. 310*), working on *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scribner, says:

> When the infection hypha enters the cavity of the cell, the protoplasmic contents of the latter apparently flow toward the hypha and collect around it. Movement of nuclei similar to that found by Blackman and Welsford in the bean cell invaded by *Botrytis cinerea*, has never been observed in this case.

Boyle, studying infection by *Sclerotinia libertiana* Fuckel (*8*), says:

> Meanwhile the nuclei of the palisade cells beneath the point of attack move toward the top of the cells . . .

An interesting side light is shed on this motion of living protoplasm toward the source of trouble by a comparison with the older work on chemotaxis and traumataxis of the nucleus. Ritter (*31*), working with the living epidermis of onion bulb scales, found that in response to a wound the nucleus and part of the cytoplasm of near-by cells flow to the side of the cell nearest to the wound. He believed that the nucleus was passive, being carried by the flowing cytoplasm. This reaction, known as traumataxis, is seen first in the cells nearest the wound, later in cells farther and farther away. The reaction weakens with distance. Later there is recovery, first near the wound and later in the more distant regions. Burning produces the same effect as pricking or cutting. Plasmolysis with sugar solution before wounding does not inhibit the reaction. Chemicals produce a reaction (chemotaxis) similar in all respects but slower. Positive chemotaxis was observed in response to a great variety

of salts, bases, organic acids, and carbohydrates. There was no response to inorganic acids nor to some organic compounds. Unrediniospores of *Puccinia porri* germinating on the surface of a bulb scale induced positive chemotaxis in the cells below.

Nestler (*27*) obtained positive traumataxis in a wide variety of plants.

Sie wurde bei Monocotylen, Dicotylen und Algen beobachtet und kommt in analoger Weise bei Blatt-Stengel-und Wurzelorganen vor.

So, in the flowing of living protoplasm towards the fungus in Mindum and in the other cases cited, we are dealing with a reaction common to many plants and capable of being elicited by a great variety of stimuli.

There is another angle from which this question can be viewed. The protoplasm of the host moves towards and surrounds the haustorium. Both the cytoplasm involved and the haustorium die. This process may be remotely akin to phagocytosis. Kolmer (*20*), speaking of the ingestion of the bacteria, in an infection, by the phagocytes, says, (*p. 181*):

As a rule, chemical stimuli serve to attract cells to the site of infection, thus constituting what is known as positive chemotaxis.

Again (*p. 183*)—

In bacterial infections the toxins, and especially the protein of dead microorganisms, are regarded as mainly responsible for the occurrence of positive chemotaxis.

And further (*p. 184*)—

Thus Metchnikoff has asserted that leukocytes might, after a time, be attracted toward substances that would kill them. Therefore, while leukocytes will migrate freely toward substances that would kill them, they may be destroyed before they reach the inflammatory area, or, having reached there, are promptly destroyed and pass into solution.

Of course, the differences between phagocytosis and conditions in rust parasitism are great. We are not even dealing with a free motile cell. The mechanics of the motion toward the invader may be different. The surface tension theory of phagocytic motion might possibly be applicable to the motion of the nucleus, but it would be difficult to apply it to the flow of cytoplasm in a cell with fixed boundaries. This much they have in common, however, whether it be significant or not: The host substance moves towards the foreign organism and the toxin emanating from it and flows around it, even though killed by it.

This toxin which proves fatal to Mindum protoplasm probably is secreted by this same rust in the other hosts, but it produces very different results there. Occasionally the nucleus is found alongside of a haustorium in Kanred, and there may be an initial flow of cytoplasm toward the haustorium, but, if so, it is inconspicuous and there is later a recovery from it. When form XIX grows on Kanred (type of infection o) the host nucleus often is near the fungus.

The nuclei in infections in Kanred and Baart increase greatly in size. This was not so marked in Mindum, for the nuclei near the fungus are killed, but farther out there is slight expansion. Eriksson (*11, pt. II*) noted nuclear enlargement in *Puccinia dispersa* Erikss. in young infections on rye:

Der kern zeigt sich etwas vergrössert . . .

And later—

Noch stärker zeigt sich indessender Hypertrophie des Kerns in der letzten Einlegung . . . unmittelbar vor dem Hervorbrechen der ersten pusteln . . .

And—

solche Hypertrophien auch weit von mycelienfäden entfernt vorkommen.

The same "hypertrophy" of the nucleus was noted in *Puccinia graminis* on oats. Eriksson believed that these enlarged nuclei represent combined host and fungous protoplasm. Magnus (*23*) finds striking enlargement of nuclei in the orchid tissues infected by an endotrophic mycorrhiza.

Ritter, in his studies of traumataxis of nuclei (*31*), found that in any cell showing the maximal reaction (i. e., where the cytoplasm and nucleus banked up on the side of the cell nearest the wound) the nucleus was above normal in size. Careful measurements were made of nuclei in a considerable area about the wound. The increase is greatest near the wound and is gradually less marked as the distance from it increases. Nestler (*27*) also noted the increase in nuclear size in tissues adjoining wounds. In an extreme case the diameter increased from 10 μ to 24.6 μ. In the later stages of the reaction the size may return to normal. In some tissues this reaction is associated with nuclear and cell divisions, a renewal of growth that helps to heal the wound. He connects the increase in nuclear size with the altered metabolic relations (Ernährungsverhältnisse).

The expansion of the nucleus, then, is another common reaction which may occur under a variety of circumstances, and may indicate renewed or increased activity.

It would be impossible to say with any certainty how many and what forces are at play in the production of the changes observed in host nuclei and plastids in the infected areas. Not all types of reaction to the rust have been studied. Any hypothesis at present must be tentative and incomplete.

According to current belief, the nucleus is intimately concerned with the metabolic activities of the cell, and it may be that the increase in the size of the nucleus can be taken as an index of its heightened activity and that the condition of the plastids is more or less closely correlated with that of the nucleus. The fungus makes heavy demands for food (in susceptible hosts at least) upon the tissue it invades. Just how the extra burden imposed by the fungus upon a cell induces the production of additional food by that cell is hard to say. At any rate, at the center of the infection in Baart, where the demands of the fungus are greatest and where also the nuclei enlarge most rapidly, the reduction in size of the plastids is soon checked and a balance of forces is struck and maintained until the nuclei collapse. Farther out, at the margin of the infection and the area just beyond it, the nuclei respond more slowly, and there the plastids show extreme reduction (cf. 10a and 10b in Table I). A little later, however, the nuclei of these same outer areas expand (just before their collapse) and we find correspondingly a partial recovery in size of plastids in these areas. Tallying with this are the facts already mentioned, that between two uredinia, in areas affected by both fungi, the nuclei enlarge sooner, and here, too, we do not find the extreme reduction in plastid size.

In Kanred nuclear expansion is proportionally quite as great as in Baart, and the response comes sooner in the outlying portions of the infection. Corresponding to this, the reduction in plastid size is more uniform throughout the infection, and, so far as observed, is not extreme.

In Mindum the relationships are different. The haustoria are supposedly secreting the same substance or substances into the host cells that they did in the other cases, but Mindum protoplasm differs in some fashion in the nature, the concentration, or the organization of its chemicals, and instead of being stimulated to greater activity, it is killed

outright. There is widespread plasmolysis, the nuclei collapse sharply, and the plastids disappear. Farther away, where the secretion of the fungus is more dilute, the host nuclei live and even undergo slight expansion.

It would seem, then, that the fungus in some fashion stimulates its host to greater metabolic activity; that the enlargement of the nucleus is an index of this increased activity; that where the nuclei enlarge most, the cell, and particularly the plastids, are least impoverished; and that the metabolic products of this heightened activity help to meet the needs of the fungus.

SUMMARY

Baart is susceptible to specialized forms III and XIX of *Puccinia graminis tritici;* Kanred is susceptible to form III and immune from form XIX; and Mindum is immune from form III and resistant to form XIX.

Appressoria of both forms of rust secrete some substance which penetrates the walls of the guard cells on which they lie and spreads through them, sometimes reaching the next cells. This substance softens the cell walls and kills the cell contents. It produces the minimum of injury to the stomata of Baart, is intermediate in its effect on Kanred, and strongest in its action on Mindum.

This effect upon the stomata seems to be independent of susceptibility and immunity, for the two forms are nearly equal in their effect on the stomata of any given host, yet differ markedly from each other in their ability to infect these hosts.

This secretion by the appressoria causes a softening of the guard cell walls and the death of the cell contents. With either of these effects the mechanism of the stoma presumably would fail and the stoma would remain closed. The entry of the fungus usually takes place before this alteration of the guard cells becomes pronounced.

The percentage of entries in Kanred varied from 5 to 18, with a general average of 13. In the other hosts it ranged from 23 to 78.

The peculiar behavior of wheat stomata, as observed by Loftfield, probably explains to some extent the partial exclusion of the fungus.

Form III develops normal haustoria in Baart and Kanred and obtains food for growth. Where the mycelium is densest, its demands upon the host for food are greatest, and its secretions into the host cells are most concentrated, the host cells are stimulated to increased metabolic activity. The nuclei increase in volume several fold. The plastids first decrease in size, but with the increase in the activities of the cell, this reduction is checked and a balance of forces is struck and maintained.

In the outlying regions of the infection in Baart the stimulus comes later and is weaker. At first the activity of the host cells is not increased, their nuclei do not expand, and the reduction in the size of their plastids is not checked. Consequently, the plastids become far smaller than those at the center of the same infection. Later there is an expansion of the nuclei in these outer regions and a partial recovery in the size of the plastids. In Kanred the marginal regions are stimulated sooner and the reduction in plastid size is less extreme.

Still later the host nuclei throughout the infected area collapse.

Form III also forms haustoria in Mindum. When the young fungus forms a haustorium in a mesophyll cell, the living contents of that cell flow rapidly to the haustorium, condense around it, and die, and the

whole cell collapses. The haustorium remains small, dense, and rounded, and soon dies. When haustoria form in an epidermal cell they may expand and function for a time, but ultimately succumb.

Each attempt of the fungus to make a haustorium wastes some of its substance. Its diminishing amount of living matter is continually transferred to the growing tips, leaving the older hyphae empty.

Older enfeebled fungi elicit less violent reactions in the host cells, but here, too, some of the cytoplasm moves to the haustorium and surrounds it, and the nucleus is to be found near by. Here, too, the haustorium and the host protoplasm near it die, but the host cell does not collapse.

Host tissues in Mindum for some distance around the fungus are plasmolyzed and an occasional cell wall is greatly swollen.

Near the fungus, nuclei die and plastids become smaller and disappear. Farther out, the nuclei live and even expand slightly, and the plastids persist.

Form III evidently secretes substances into the host cells. Baart and Kanred tissues are stimulated and produce additional food that meets the needs of the fungus. Mindum tissues, on the contrary, are killed outright by the more concentrated solution of this substance. The outer regions of the infection in Mindum are slightly stimulated, but it is not clear whether this is due to a dilute solution of the same toxin that killed the central cells or to secondary substances which have formed in the dying cells and diffused out from them.

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PLATE 1

Puccinia graminis tritici, form III on Baart

A.—Seven days after inoculation. Longitudinal section of stoma bearing appressorium. Central part of guard cell dying. × 730.

B.—From infection seven days old. Haustorium at *a* and hyphae at *b* nearly empty. × 1130.

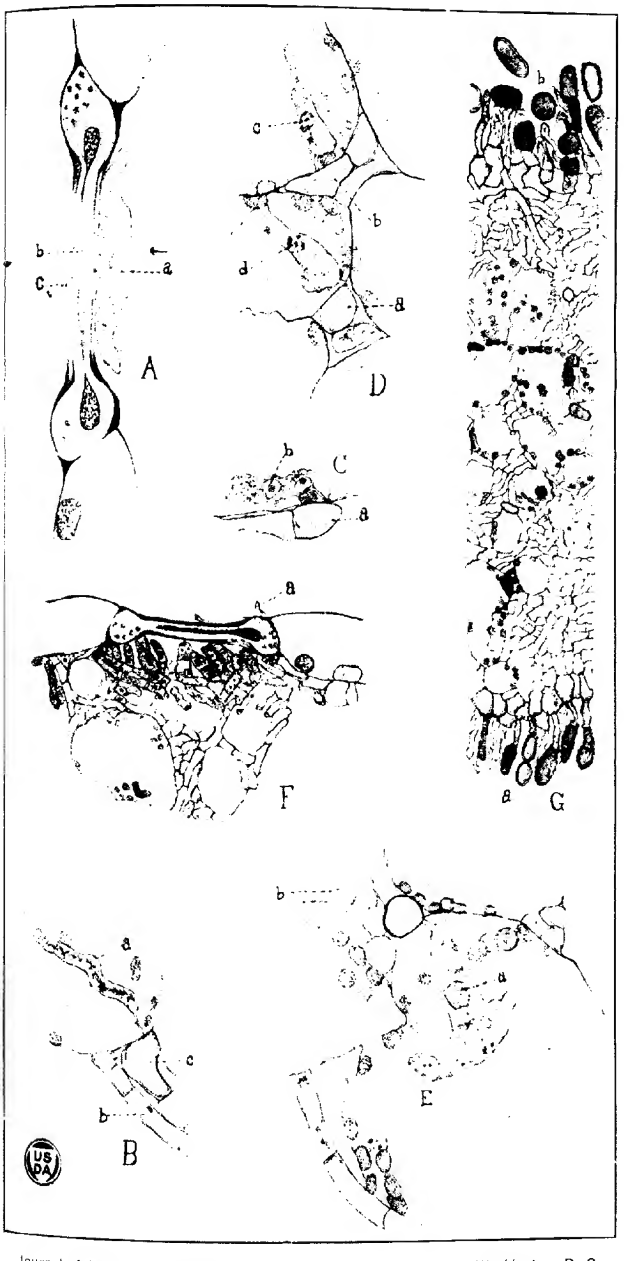
C.—Seven-day infection. Haustorium at *b* showing nuclei. Modified haustorium mother cell at *a*. × 1130.

D.—Seven-day infection. Haustoria at *c* and *d*, connected by narrow necks to the modified mother cells at *b* and *a*. × 1130.

E.—Ten-day infection. Nearly empty hyphae at *b*, and partly drained haustorium at *a*. × 1130.

F.—Seven-day infection. Portion of young uredinium forming under a stoma. Young spores and their stalks dense in cytoplasm, while the mycelium farther back is drained. × 333.

G.—Fourteen-day infection. Narrow strip through the center of an infection bearing spores on both surfaces of the leaf. The heavy mycelium is drained throughout. Host cells often are crowded out of shape or even obliterated. × 333.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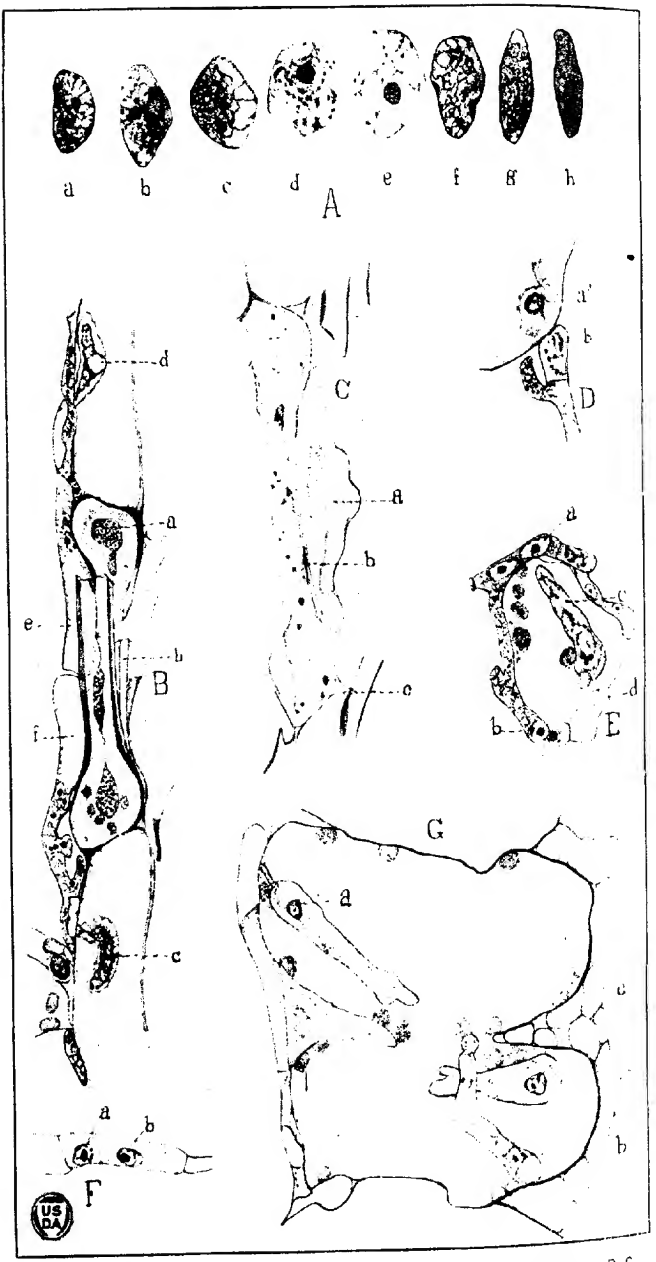
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PLATE 2

Puccinia graminis tritici form III on Baart (A) and Kanred (B–G)

A.—Baart. Series showing alterations of nuclei in infected area. × 1130.

a. Normal nucleus from uninfected tissue.

b, *c*, and *d*. Nuclei from seven-day infection, showing progressive expansion of nuclei, increase in nucleoli and loosening of chromatin net.

e. Maximum size, 10-day infection.

f, *g*, and *h*. Progressive collapse of nucleus. Ten- and 14-day infections.

B.—Kanred. Four-day infection. Two appressoria at one stoma. Each produced a substomatal vesicle (*e* and *f*). The infecting hyphae grew in opposite directions and each produced a haustorium, *c* and *d*, in an epidermal cell. Outer guard cell wall modified at *b*. × 730.

C.—Kanred. Seven-day infection. Guard cell killed by appressorium of fungus *a*. Adjoining epidermal cells weakened and broken. × 730.

D.—Kanred. Seven-day infection. Young haustorium *a* forming from the mother cell *b*. × 1130.

E.—Kanred. Seven-day infection. Cell surrounded by vigorous hyphae. Large haustorium *c* from mother cell *d*. × 1130.

F.—Kanred. Nine-day infection. Fungus cell from mycelium below uredinium. Cytoplasm nearly gone. Nuclei distinct. × 1130.

G.—Kanred. Eleven-day infection. Mesophyll cell from region where mycelium is nearly empty. Haustoria at *a*, *b*, and *c* also partly drained. Nucleus in haustoria distinct. × 1130.

PLATE 3

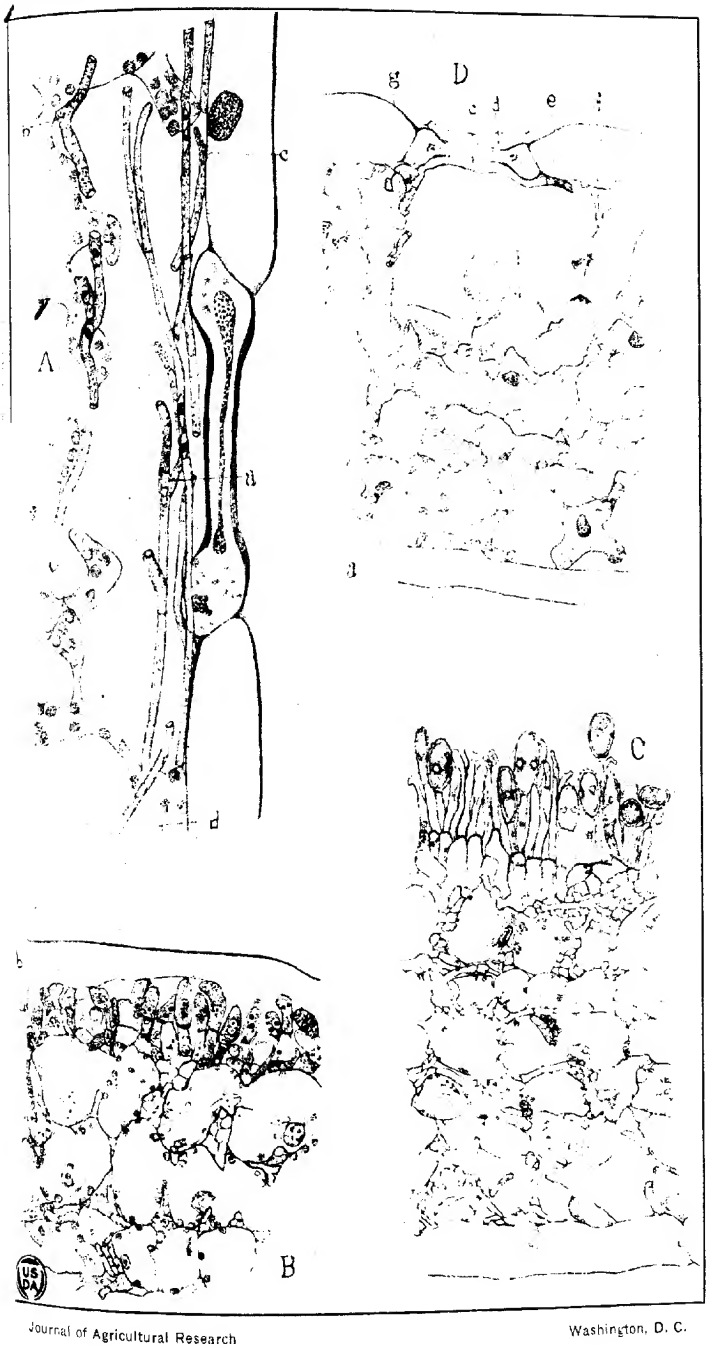
Puccinia graminis tritici form III on Kanred (A–C) and Mindum (D)

A.—Kanred. Seven-day infection. Group of "runners" of the fungus growing along the inner surface of the epidermis through a substomatal chamber. × 730.

B.—Kanred. Seven-day infection. Portion of young uredinium and mesophyl tissue below it. Spore bearing layer *a* lifting epidermis *b*. Cell contents of centra mycelium drained into uredinium. × 333.

C.—Kanred. Fifteen-day infection. Strip through a longitudinal section of the leaf at the center of an old uredinium. Epidermis is gone and many spores liberated Mycelium practically empty. × 333.

D.—Mindum. Four-day infection. Young infection. Appressorium *d* and substomatal vesicle below it, *c*, empty. First host cell attacked by the fungus *f* dead and shriveled. Second attack begun at *g*. Several cells plasmolyzed. × 333.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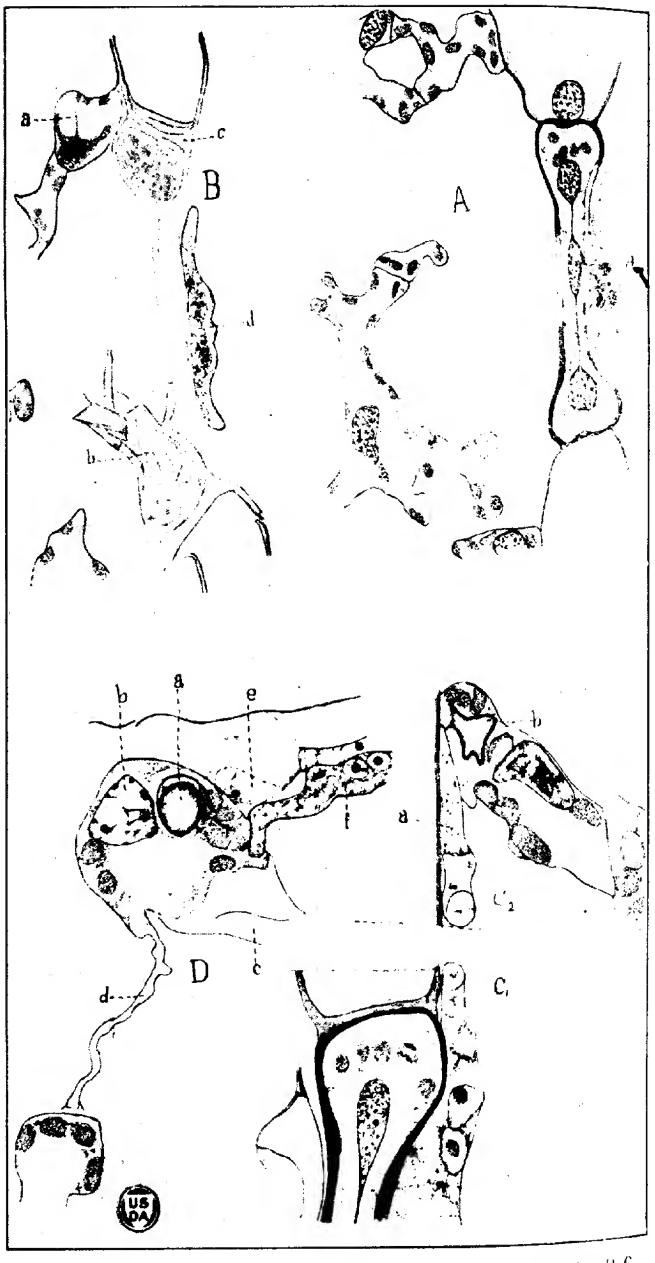
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PLATE 4

Puccinia graminis tritici form III on Mindum

A.—Three days after inoculation. Longitudinal section of stoma bearing appressorium. Both outer and inner walls of guard cells modified. ×730.

B.—Seven days old. Appressorium *d* dying, guard cells dead, adjoining walls of epidermal cells swollen, and nearest mesophyll cell *a* dying. The fungus has not entered. ×730.

C.—Two-day infection. Two sections. Head of stoma and part of infecting hypha in C_1; rest of hypha, haustorium mother cell *a* and young haustorium *b* in C_2. Plastids and nucleus of host cell collecting around haustorium. ×1460.

D.—Two-day infection. Slightly later. Haustorium mother cell at *e* collapsed and dying. Nucleus and cytoplasm of host cell concentrated about haustorium *a*, leaving two lobes of the cell *c* and *d* empty and collapsed. ×1460.

PLATE 5

Puccinia graminis tritici form III on Mindum

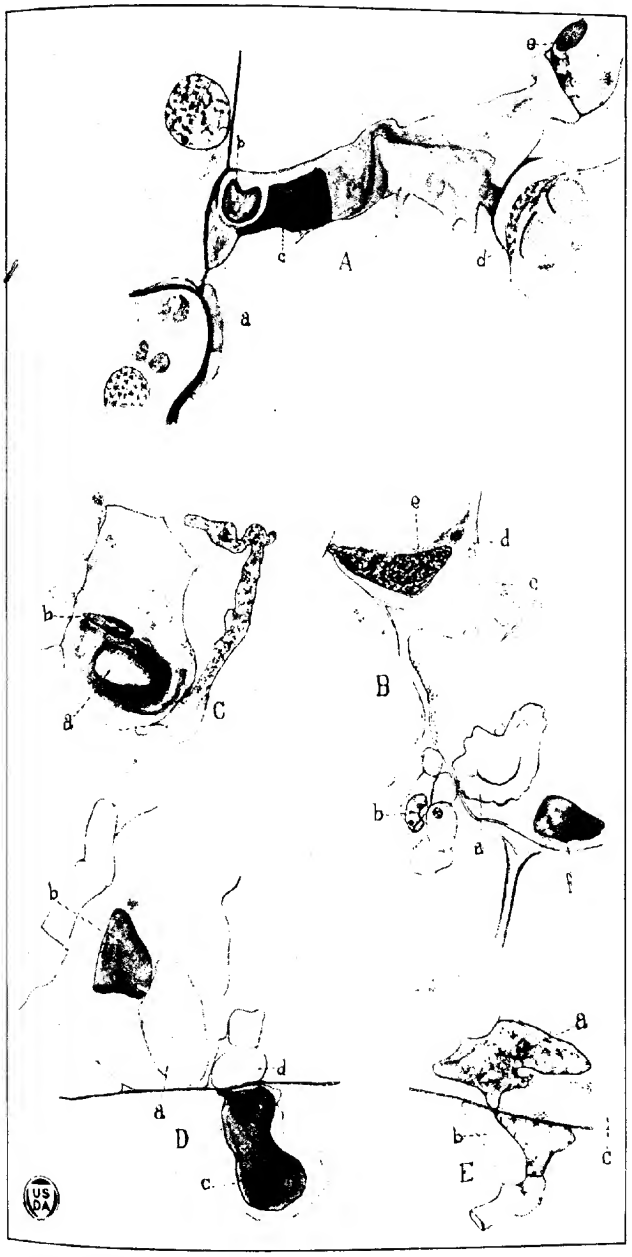
A.—Two-day infection. Dead infecting hypha *a* at head of stoma. Attacked cell dying and adjoining cells *d* and *e* harmed. Dead haustorium at *b* and nucleus *c* pressed against it. ×1460.

B.—Seven-day infection. Group of hyphae at *b*. The body and neck of the haustorium *a* and the heavy layer coating it are dead. Adjoining cells at *c* and *e* are plasmolyzed and the wall at *d* is swollen. ×1460.

C.—Seven-day infection. Dead haustorium *a* with cytoplasm of host cell concentrated about it in layers. Nucleus *b* dead and flattened. ×1460.

D.—Eleven-day infection. Two haustoria from the mother cells at *d*. One in a dead mesophyll cell at *a* is nearly dissolved. Nucleus is at *b*. The second, *c*, is an epidermal cell, shows some lamination, and is covered by a transparent irregularly laminated sheath. ×1460.

E.—Seven-day infection. Large, nearly normal haustorium in an epidermal cell.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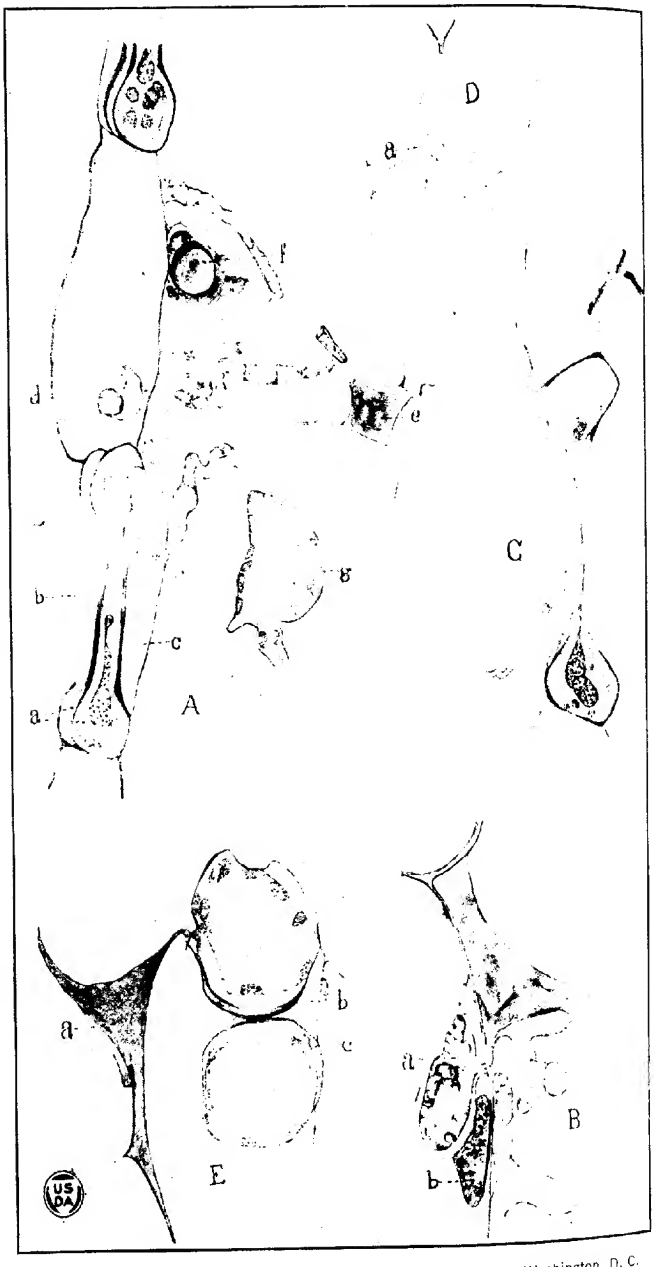
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PLATE 6

Puccinia graminis tritici form III on Mindum

A.—Seven-day infection. Portion of infection showing appressorium at *b*, part of the first haustorium *d*, dead cell at *e*, and dying cell with haustorium at *f*. ×730.

B.—Another part of same infection with haustorium *a* in an epidermal cell. It is living but is heavily coated, and the host nucleus *b* is in attendance. ×1460.

C.—Seven-day infection. Stoma occupied by fungus and showing guard cell walls greatly swollen. ×730.

D.—Seven-day infection. Greatly swollen walls in another infection close to that in Plate 6, A. ×1130.

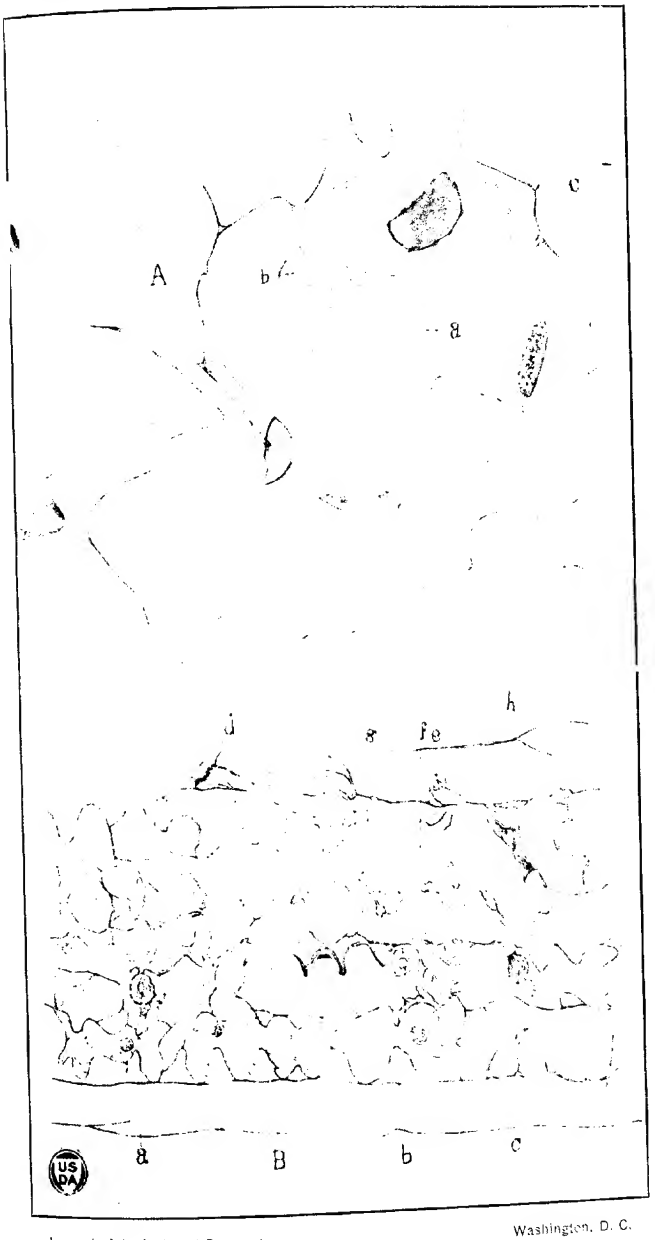
E.—Four-day infection. Walls beginning to swell at *b* and *c* near dead cell *a*. ×1130.

PLATE 7

Puccinia graminis tritici form III on Mindum

A.—Eleven-day infection. Older infection with an occasional wall greatly swollen and showing the layers of which it is composed. ×1460.

B.—Fifteen-day infection. Portion of infected leaf with dead stoma at *d*, a few scattered hyphae, and coated dead haustoria at *a*, *b*, *c* and *e*. Host cells first attacked, *f*, *g* and *h*, are dead and collapsed. Cells attacked later are empty but retain their shape. Occasional swollen walls. ×333.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THE INTRACELLULAR BODIES ASSOCIATED WITH THE ROSETTE DISEASE AND A MOSAICLIKE LEAF MOTTLING OF WHEAT[1]

By HAROLD H. MCKINNEY, *Pathologist*, SOPHIA H. ECKERSON, *Microchemist*, and ROBERT W. WEBB, *Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

It is the purpose of this paper to describe briefly the intracellular bodies found in wheats affected by the rosette disease and the mosaic-like leaf mottling. The literature bearing on the problem will be reviewed in another publication.

In a recent abstract by the writers[3] and in a paper by McKinney[4] attention was called to unusual intracellular bodies which appeared to be associated with the rosette disease of wheat (Pl. 1). It was pointed out in these publications that the intracellular bodies are also associated with a mosaiclike leaf mottling occurring on wheat plants which may or may not show the rosette symptoms (Pl. 2), and it was also pointed out that it is not definitely known whether wheat rosette and the leaf-mottled condition are different responses to the same causal agent or whether they are due to separate causes. Observations made on field experimental plots conducted at Granite City, Ill., during 1923 show certain relationships between the two manifestations which suggest that they may be due to one causal agent. This correlation seems even more striking than the correlation[5] which is sometimes noted between the rosette disease and the occurrence of *Helminthosporium sativum*.

Although the leaf mottling in wheat is typical for the mosaic diseases of the Monocotyledons, there are indications that it behaves somewhat differently from these latter diseases in that the causal agent for the leaf mottling of wheat appears to be carried over from year to year in the soil. In field experiments conducted in 1923 at Granite City, Ill., and Madison, Wis., with heavily infested soil, rosette and leaf mottling occurred on from 95 to 98 per cent of the wheat plants of a susceptible variety (Harvest Queen). When such infested soil was disinfected with formaldehyde or steam and subjected to the same conditions as the infested soil, rosette and leaf mottling apparently were completely absent throughout the entire growing season. This control was effected at Granite City, Ill., even though the apparently healthy plants were surrounded by thousands of wheat plants showing an abundance of leaf mottling. In addition, flying insects, especially aphids and chinch bugs, were abundant during certain periods.

[1] Accepted for publication Nov. 1, 1923.

[2] These investigations have been carried on in cooperation with the Wisconsin and Illinois Agricultural Experiment Stations.

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[4] MCKINNEY, Harold H. INVESTIGATIONS OF THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL. *In* Jour. Agr. Research, v. 23, p. 771-800, 2 fig., 8 pl. 1923. Literature cited, p. 799-800.

[5] ——— THE SO-CALLED TAKE-ALL DISEASE OF WHEAT IN ILLINOIS AND INDIANA. (Abstract.) *In* Phytopathology, v. 11, p. 37. 1921.

Like the rosette disease[6], the leaf-mottled condition has not yet been readily reproduced under artificial conditions, and for this reason it has not been determined whether we are dealing with a true virus disease or whether the causal agent is transmitted by some soil insect or other soil organism of animal nature.

The varietal ranges of the rosette and the leaf-mottled conditions are of interest at this point. Out of 104 winter-wheat varieties and selections, grown on soil naturally infested with the rosette and leaf mottling causal agent or agents, only 9.6 per cent of the varieties or strains showed definite rosette, whereas 86.5 per cent showed leaf mottling in varying degrees of severity. In all cases varieties or strains showing definite rosette also manifested a definite leaf mottling. The small proportion of varieties and strains which are susceptible to the rosette disease is unusual among plant diseases, and this relationship suggests a rather strong possibility that rosette may be a severe manifestation of a malady which has a wide varietal range and of which leaf mottling may be a milder expression.

DESCRIPTION OF INTRACELLULAR BODIES

Microscopic studies of both fresh and embedded material from the tissues of field-grown winter-wheat plants affected by the rosette disease have shown that certain cell inclusions are present in the crown tissue in the late winter and early spring. As the disease progresses, the bodies become more numerous and more generally distributed throughout the tissues of the plant. While the bodies are known to occur in the roots, throughout the crown tissue, in the leaf sheaths, and in the leaves, further studies may reveal them in other parts of the plants. As yet the bodies have not been found in plants known to be free from rosette or leaf mottling.

In preparations from material killed and fixed in the usual botanical fixatives and stained with Flemming's triple stain, the intracellular bodies show a marked affinity for orange G. The bodies have shown only a slight affinity for safranin and much less for gentian violet. When preparations are stained with Heidenhain's iron-alum haematoxylin the bodies tend to take the stain less intensely than the host nuclei, and in the destaining process the bodies generally lose the stain much sooner than the nuclei.

The bodies usually occur singly in the host cells. Occasionally two or three are found in the same cell, but this seems to be the exception rather than the rule. Frequently the bodies are more abundant in tissues adjacent to internal lesions (Pl. 3, B and Pl. 4, B) of the crown tissue. Usually crown tissue containing intracellular bodies is of a yellow or yellowish-brown color even though definite internal lesions may not be present.

In form, the bodies vary greatly. Round to oval are perhaps the most common forms, but it is not unusual to find bodies rather irregular in shape, as shown in the several plates. In long host cells it is common to find very long bodies such as the one shown in Plate 8, fig. 7.

In size, the bodies range from much smaller to considerably larger than the host nuclei. In the case of bodies less than 2 to 3 microns in size, it is difficult to be certain of their exact identity. It is believed, however,

[6] McKinney, Harold H. INVESTIGATIONS OF THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL. In Jour. Agr. Research, v. 23, p. 771-800, 2 fig., 8 pl. 1923. Literature cited, p. 799-800.

that they may be a micron or less in size in their earliest stages, since globules of these smaller dimensions may be seen in some cells. Studies made thus far indicate that the size of the bodies increases with the age of the host cells including them. Further, typical bodies which can be definitely identified have not yet been observed in the very young cells of the young central and lateral buds. The minute bodies which are suggestive of an early stage of the large bodies have been found in cells a little distance back from the youngest cells, and from this latter region back into the older cells, the bodies seem to increase gradually in size until the large sizes are reached in the oldest cells of the leaf sheaths and crown.

The bodies occur in various relations to the cell nuclei as shown in Plates 3 to 8. In the majority of cases the bodies occur either free from or in more or less close contact with the nucleus. Occasionally, however, they may be found partially or completely surrounding it, as shown in Plate 7, Figs. 1, 3, 4, and 9.

The contents of the bodies seem to be of a rather homogeneous structure containing many large and small vacuoles. The large vacuoles usually are very conspicuous when viewed through 4 mm. objectives, but the small ones are visible only when high resolving lenses are used. Studies made with carefully stained sections from killed and fixed tissues indicate that the bodies are surrounded by a membrane and there is a strong suggestion that they consist of alveolar protoplasm.

The majority of the intracellular bodies in wheat studied thus far show no detail in their vacuoles. However, in a few cases in both fresh and fixed materials, these vacuoles have contained granulelike and also elongated bodies. Structures suggesting nuclei have occasionally been found (Pl. 8, Figs. 4, 5, and 8), but these are neither consistent nor definite in the material studied. In many fixed and stained preparations, the vacuoles are surrounded by densely staining rings as shown in the various plates. As yet the intracellular bodies have not been observed to possess definite independent movement. In fresh, unstained material they have been observed to move from place to place in the cell, but this movement was attributed to the distinctly evident streaming movement of the surrounding cytoplasm of the host cells.

Examinations of fresh material in sterile water mounts have occasionally shown moving granulelike bodies and also elongated, flexible bodies in the vacuoles of the larger intracellular bodies as shown in Plate 6. These intravacuolar bodies usually are in motion when first examined and remain so for periods of from 36 to 42 hours. Then all movement seems to stop. The granules and elongated bodies have been noted only occasionally, but it has been rather evident that, when a few are found, many others may be discovered in the same plant and to some extent in other plants grown under the same conditions. A few structures resembling those in the vacuoles of fresh material have been found in fixed and stained material, but it has not been possible to determine their exact nature. In the specimens of fresh material, the movement of the granulelike bodies could be interpreted as typically Brownian. The movement of the elongated bodies, however, seems to differ from the ordinary Brownian type. The movements of these latter forms are more like those which have been described for the mitochondria.

From the studies made thus far the majority of the wheat cells containing intracellular bodies show no marked differences from the cells

free from the bodies, and the host nuclei seem to show little or no abnormality when the bodies are in the cells. Further light may be thrown on these points when the relation between the intracellular bodies and the internal lesions, described earlier, is determined.

The intracellular bodies in wheat are similar to certain of the intracellular bodies associated with other plant diseases and with certain animal diseases, but they differ in a number of particulars from certain others which have been described in diseased tissues. These comparisons will be taken up in a later paper.

POSSIBLE NATURE OF THE INTRACELLULAR BODIES

The studies made to date show clearly that the bodies in question are not artifacts and that their nature is such that they do not yield readily to definite interpretation. While it is possible that the bodies may be organisms, it is also possible that they are the result of the reaction of the host cells to the disease. A rather comprehensive study of the literature shows that there are several possibilities in connection with the latter interpretation, some of which are more plausible than others, but a considerable amount of comparative study must be made before these interpretations can be definitely accepted or rejected.

While it is recognized that the intracellular bodies associated with wheat rosette and leaf mottling may be a stage of some definite parasite, it is also recognized, on the basis of the cytological studies made thus far, that the distribution of the intracellular bodies in the host tissue and their apparent parallel development with that of the host cells do not seem to conform exactly with the distribution and development of any plant parasite known.

In general, the intracellular bodies in question resemble the cell inclusions of unknown nature which are associated with some of the virus diseases of animals. This resemblance is especially striking in connection with certain of the Negri and Guarnieri bodies which are associated with rabies and smallpox, respectively.

PLATE 1

A.—Winter-wheat plants (Harvest Queen variety) showing the rosette disease. These plants were grown outdoors in naturally infested soil.

B.—Healthy Harvest Queen wheat plants grown under exactly the same conditions as those shown in A except that the infested soil was sterilized with steam just before the seed was sown.


A
B

Intracellular Bodies in Wheat Rosette

PLATE 2

PLATE 2

A.—Harvest Queen wheat plant showing the rosette disease and a mosaic like leaf mottling.

B.—Leaf from a healthy wheat plant of the same variety.

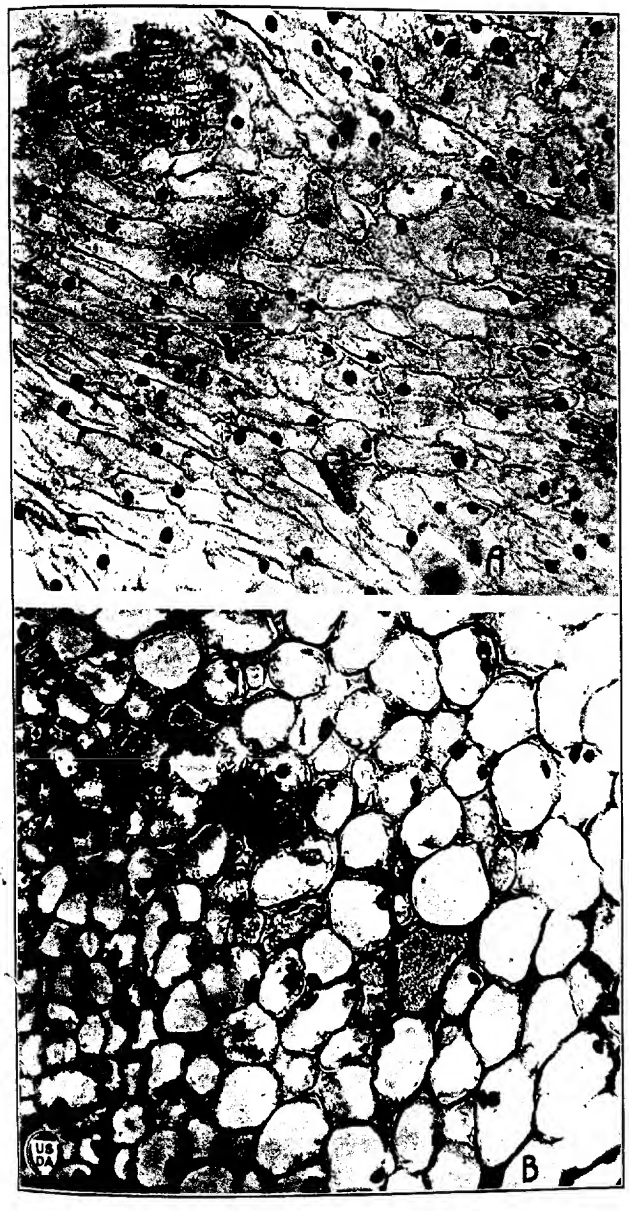
71689—24——4

PLATE 3

Photomicrographs from longitudinal sections of the tiller bases and crown of healthy and rosette-diseased Harvest Queen wheat plants. Material was killed and fixed in weak chrome-acetic fluid and stained with Heidenhain's iron-alum haematoxylin. × 277.

A.—Crown tissue from healthy plant.

B.—Crown tissue from rosette-diseased plant. Note the cells containing intracellular bodies in addition to the nuclei and also the granular nature of the cells which have become necrotic.


A
B


A
B

PLATE 4

A.—Photomicrograph from a longitudinal section of a leaf sheath from a rosette-diseased Harvest Queen wheat plant. Material killed and fixed in weak chrome-acetic fluid and stained with Heidenhain's iron-alum haematoxylin. The intracellular bodies are marked x; all other bodies are nuclei. × 556.

B.—Photomicrograph from a longitudinal section of a tiller base from a rosette-diseased Harvest Queen wheat plant. Material killed, fixed, and stained same as A. Note the necrotic cells and their granular nature. × 556.

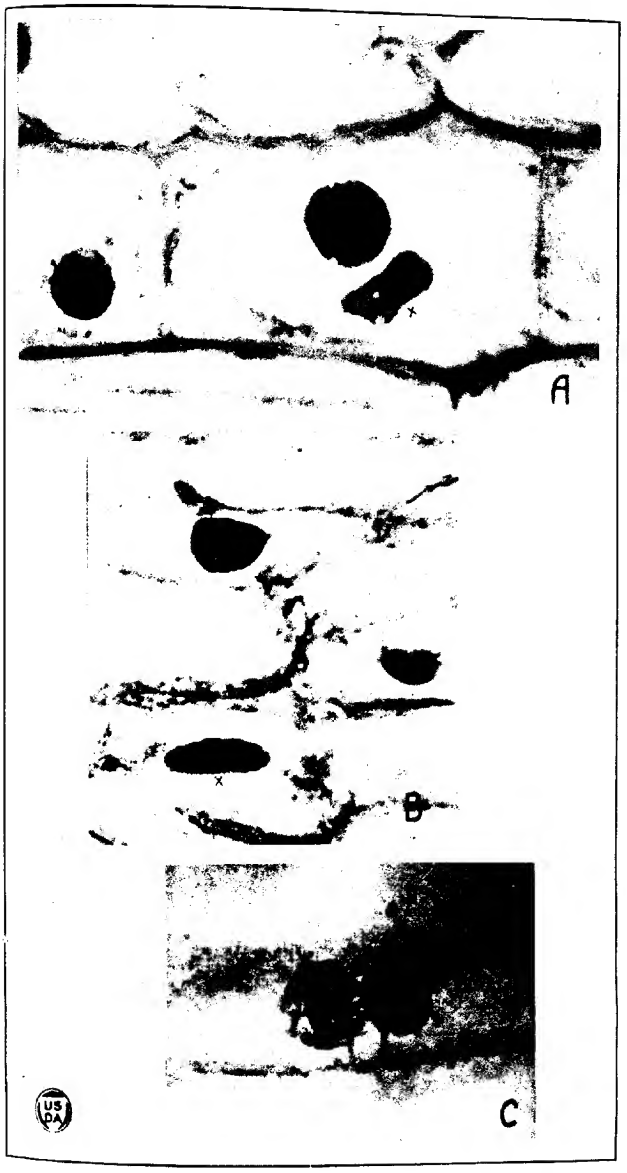
PLATE 5

Photomicrographs showing different types of intracellular bodies in rosette-diseased Harvest Queen wheat. Fixation same as for the material illustrated in Plates 4. The intracellular bodies are marked XIIII.

A.—An irregular-shaped body showing pseudopodia like projections. It is very common to find the vacuoles surrounded by a dense ring as is here shown.

B.—An elongated type of body containing elongated structures in the large vacuole. This is the body shown in the drawing in Plate 8, fig. 9.

C.—A very common type of body found in wheat tissue from plants affected by rosette or the leaf mottling. This body resembles certain of the Negri and Guarnieri bodies associated with rabies and smallpox, respectively.


A
B
C

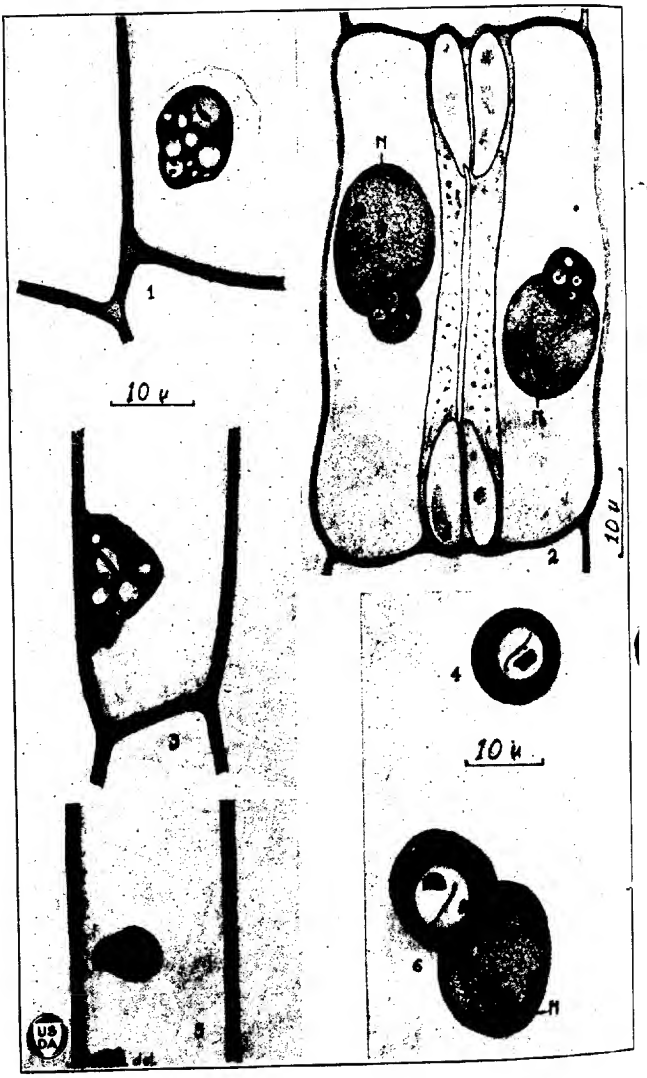

1
10 μ
N
N
2
10 μ
3
4
10 μ
5
6
N

PLATE 6

Drawings made from fresh, unstained living tissues of wheat plants.

1, 3, and 5.—Intracellular bodies in the tissues from the lower part of outer leaf sheaths. This material is from Harvest Queen wheat plants growing in rosette-infested soil under somewhat artificial conditions. The symptoms of rosette or leaf mottling had not yet developed. Note the elongated bodies and the granules in some of the vacuoles. These were all in motion. The elongated bodies were especially active, showing an eellike movement. While these movements may be all of the Brownian type this is not the only possibility.

2.—Intracellular bodies in the guard cells of a leaf stoma. Material from a mottled leaf of a Kanred wheat plant. This variety is not susceptible to rosette, but is susceptible to the mosaic like leaf mottling. Host nuclei are marked N. The granules in the vacuoles of the intracellular bodies were in motion.

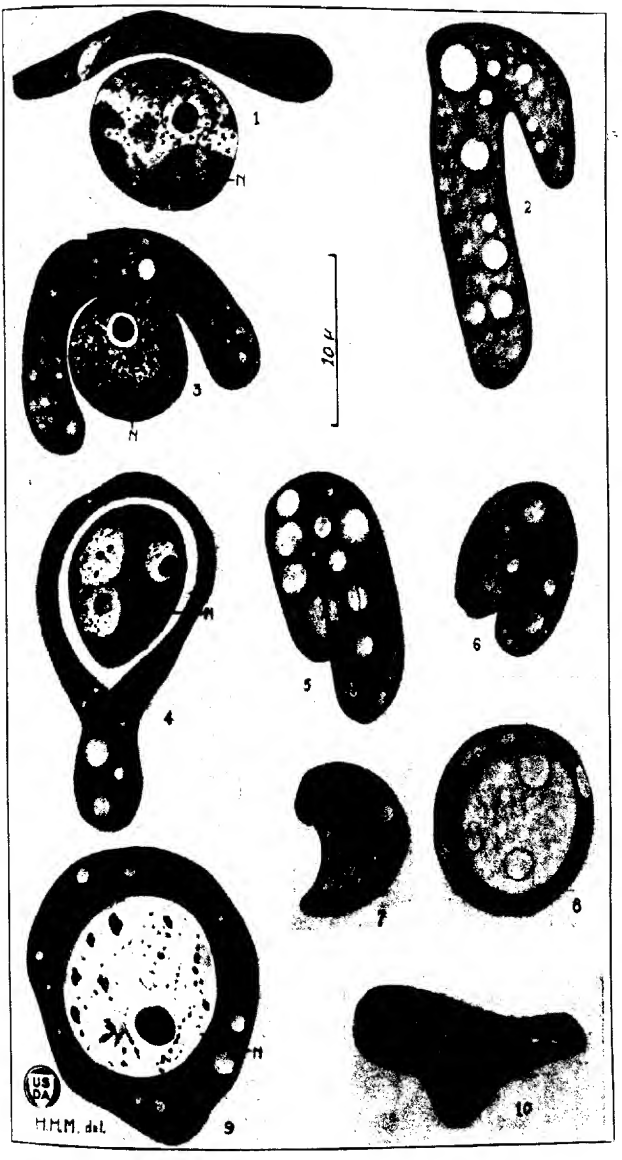
4 and 6.—Intracellular bodies in the cells of a Harvest Queen wheat leaf showing the mosaiclike leaf mottling. The plant was affected by the rosette disease. All of the bodies in the vaculoes were in motion. Those in the central vacuoles assumed many shapes and occupied many different positions in the vacuoles. The movements of the long bodies were the same as in those shown in 1 and 3. Host nuclei marked N.

PLATE 7

Drawings of intracellular bodies in tissues from the tiller bases of Harvest Queen wheat plants affected by the rosette disease.

Figures 3 and 9 from material killed and fixed in Flemming's weak solution, all other figures from preparations killed and fixed in weak chrome-acetic solution. All preparations were stained with Heidenhain's iron-alum haematoxylin. Figures 1, 3, 4, and 9 show bodies in relation to the host nuclei marked N.

The remaining figures show bodies of somewhat unusual types. Figure 8 shows an almost spherical body with an unusually large central vacuole. The matrix surrounding this vacuole contains many small vacuoles.


1
N
2
3
N
10 μ
4
N
5
6
7
8
N
USDA
H.M.M. del.
9
10

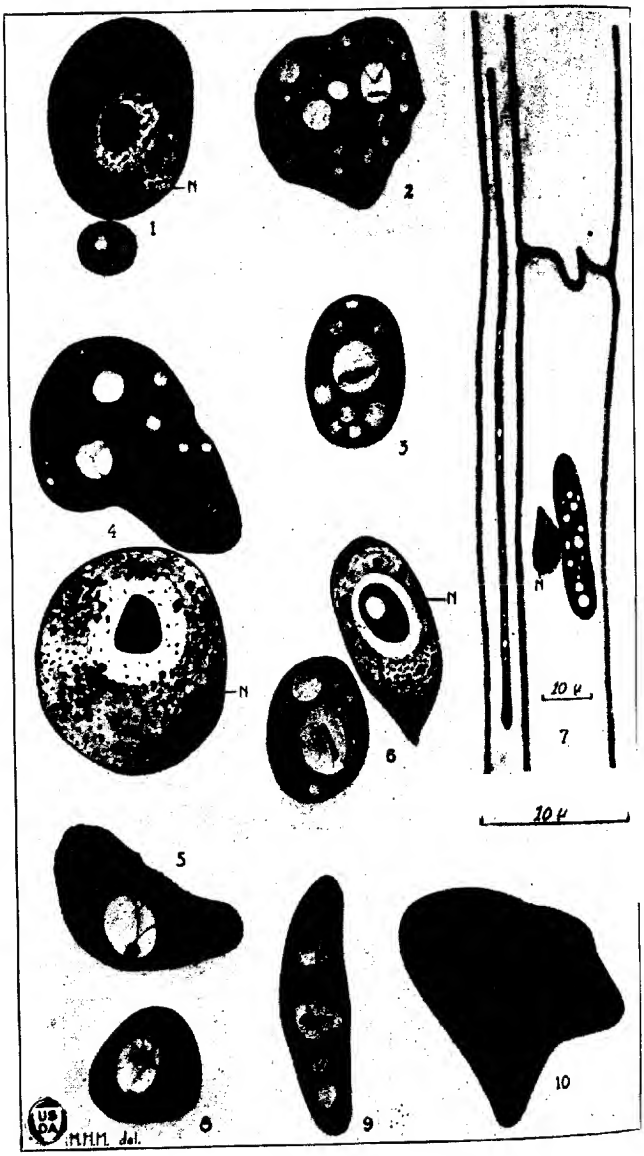

N
1
2
3
4
N
N
6
10 μ
7
10 μ
5
8
MHM. del.
9
10

PLATE 8

Drawings of intracellular bodies in tissues from the tiller bases of Harvest Queen wheat plants affected by the rosette disease. All figures from material killed and fixed in weak chrome-acetic solution. All preparations, except those in figure 6, stained with Heidenhain's iron-alum haematoxylin. Material in figure 6 stained with Flemming's triple stain.

1.—The intracellular body shown here is about the smallest stage which could be identified with certainty. Much smaller bodies occur in cells and these may be still earlier stages of the intracellular bodies. Host nucleus marked N.

2.—This body shows an extreme alveolar structure. Note the heavily stained ring around the large vacuole and the elongated structures within. This deeply stained ring occurs frequently around the vacuoles.

3, 6, and 8.—Irregularly ovoid and nearly spherical bodies most commonly found.

4.—An unusual intracellular body. Note the dark wheellike structure resembling the nuclei of certain protozoa. This is an unusual type. Host nucleus marked N.

5, 8, and 9.—These bodies show structures in the central vacuole; 9 is a drawing of the same body shown in the photomicrograph in Plate 5, B.

6.—Note the vacuolated nucleole in the host nucleus marked N. These vacuolated nucleoles can not be distinguished from small intracellular bodies in preparations stained with Heidenhain's iron-alum haematoxylin. In the case of preparations stained with Flemming's triple stain, however, the nucleoles take the safranin and the intracellular bodies take the orange G.

7.—Note the very long intracellular body. This type is common in very long cells, and can be readily distinguished from the long nuclei also occurring in these cells. No nucleus was present in the long cell here shown.

10.—This body is interesting from the standpoint of form and the very fine alveolar structure. There is also a slightly granular structure near the less dense area around the vacuole.

NOTES ON THE BIOLOGY OF THE FOUR-SPOTTED BEAN WEEVIL, BRUCHUS QUADRIMACULATUS FAB.[1]

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This report summarizes a portion of the results of bean-weevil investigations which have been conducted for several years at Alhambra, Los Angeles County, Calif. The series of weevils from which the data discussed in this paper were obtained consisted of 61 pairs of *Bruchus quadrimaculatus* Fab., including all the females and most of the males which developed from eggslaid August 15, 1919. The prolonged developmental period of the progeny of these weevils, extending as it did over the entire cool winter season, made this series particularly interesting and significant, and an analysis was made of the data from several points of view. The summary as presented deals with the effect of cool weather upon the development of the species; the effect of the age of the parent females, at the time of oviposition, upon the number and viability of the eggs and the development of the larvæ; and the longevity, preoviposition period, and egg laying of the parent insects. These data are all related to the rate of increase, and it is believed that they will contribute something toward a more thorough understanding of the life of this widespread and destructive species.

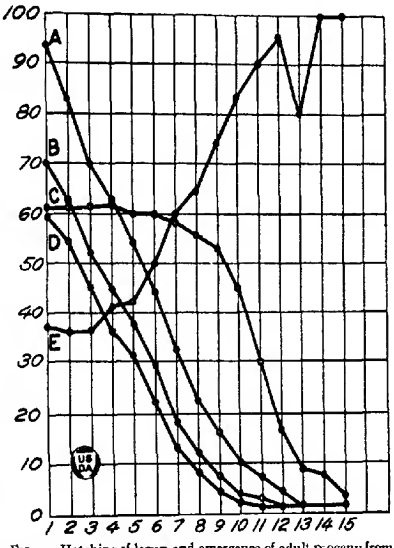

FIG. 1—Hatching of larvæ and emergence of adult progeny from eggs laid by 61 *Bruchus quadrimaculatus*: Abscissæ—laying days: *A*, eggs laid each day: *B*, hatching of eggs: *C*, number of females ovipositing; *D*, emergence of adult progeny: *E*, percentage of eggs not producing adults. *A*, *B*, *D*, one cipher omitted and figures less than 10 shown as 1.

The principal food of *Bruchus quadrimaculatus* in the bean warehouses of California is the blackeye cowpea or bean (*Vigna sinensis*), which was used in the present experiment. Eggs were deposited from September 18 to October 15 by females which emerged September 17 to October 2, and the eggs of each female were counted and removed daily to shell vials.

A survey of Table I at once suggests that the greatest number of eggs laid by a female is deposited during the early period of oviposition. Twenty-five laid their greatest number on the first day, 17 laid their greatest number on the second day, 7 on the third, 3 on the fourth, 3 on the fifth, and 6 laid the same maximum on two or more days. Figure 1 shows, however, that the total number of eggs laid daily by all the females decreased with striking regularity as the females became older. Individual records of weevils Nos. 5, 9, 17, 49, 57, 58, and others, very nearly

[1] Accepted for publication Aug. 11, 1923.

TABLE I.—*Oviposition of 61 pairs of Bruchus quadrimaculatus at Alhambra, Calif.*

	September.													October.																	Total.
	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
TEMPERATURE																																
Maximum	81	83	80	76	88	98	96	93	88	64	65	63	73	75	73	77	81	86	89	89	79	67	77	88	84	79	74	78	...	...	...	...
Minimum	54	60	59	58	50	56	51	59	60	59	57	55	45	47	51	43	43	44	49	46	61	54	47	47	50	53	49	45	...	...	...	...
Average	67	72	69	67	69	77	74	76	74	61	61	59	59	61	62	60	62	65	69	68	70	60	62	68	67	66	61	62	...	...	...	...
EGGS LAID																																
Pair 1	17	10	10	17	14	1	14	3	11	2	0	0	0	0	0	...																99
2	16	8	12	9	6	6	5	7	5	2	0	0	0	0	0	...																76
3	19	9	12	10	8	2	10	1	3	0	...																					74
4	...	12	15	20	15	5	18	8	12	4	0	0	4	2	0	0	0															115
5	...	22	15	15	14	6	13	2	11	6	0	0	2	0	0	0	0															106
6	...	19	24	5	10	8	11	7	11	7	0	3	1	...																		106
7	...	9	17	12	11	12	10	5	6	2	1	0	...																			85
8	...	...	14	11	13	8	8	12	14	8	1	3	1	0	0	0	0	0	0	...												93
9	...	...	13	10	13	10	9	6	6	3	1	0	0	0	...																	71
10	...	...	14	10	12	7	10	12	10	6	1	0	0	0	0	...																82
11	...	...	21	11	13	12	12	7	14	1	0	1	1	0	0	...																93
12	...	...	...	13	18	11	12	7	14	5	1	0	2	2	0	0	0	...														81
13	...	...	...	9	16	7	15	8	15	8	0	2	2	0	0	0	0	0	...													84
14	...	...	...	13	15	6	16	12	12	6	1	3	2	0	0	0	0	0	0	...												85
15	...	...	...	15	17	5	13	6	15	7	0	4	2	0	0	0	0	0	0	0	...											85
16	...	...	...	15	13	4	14	12	12	8	2	1	0	0	0	0	0	0	0	0	0	...										81
17	...	...	...	17	14	8	15	13	14	5	7	1	1	2	0	0	0	...														97
18	...	...	...	...	11	17	12	15	19	8	6	1	3	2	0	0	0	1	0	...												95
19	...	...	...	...	4	19	14	9	12	6	4	1	0	2	0	0	0	0	0	0	...											71
20	...	...	...	...	11	7	19	15	14	12	2	2	3	3	0	0	0	2	0	0	0	...										90
21	...	...	...	...	5	20	15	14	15	12	4	5	2	1	0	0	0	1	0	0	0	0	...									96
22	...	...	...	...	18	11	14	12	12	9	3	2	2	4	0	0	0	0	0	0	0	0	0	...								87
23	...	...	...	...	12	14	10	8	2	1	9	3	0	2	0	0	0	0	0	0	0	0	0	0	...							61
24	...	...	...	...	12	11	15	8	9	11	1	2	4	1	0	0	1	0	0	0	0	0	0	0	0	...						75

25					11	14	14	14	10	11	3	0	0	0	0	0	0															77
26					8	9	14	13	14	10	1	5	1	2	2	2	0															81
27					12	17	10	11	14	10	3	3	5	3	1	0	1	1	0	0	1											92
28					10	18	15	12	12	8	10	3	1	2	0	0	0															91
29					10	9	15	14	17	13	3	5	2	2	1	0	0															91
30					12	10	16	13	12	7	5	3	3	0	0	0	1															82
31					11	7	8	18	12	9	2	2	0	3	2	0	0	0														74
32					15	8	13	11	10	8	5	1	2	1	1	1	1	0	1													78
33					12	12	13	14	13	11	7	3	0	7	2	1	0	0	0	0	0											95
34						30	14	17	11	9	6	3	2	4	2	0	1	0	0	0	0											99
35						14	11	16	10	8	7	1	0	1	1	1	3	0	0	0												73
36						16	16	17	13	7	7	4	2	3	1	1	1	0														88
37						18	16	14	16	(a)																						64
38						8	20	15	11	12	3	5	5	2	0	0	0	0	0	0												81
39						17	19	14	13	8	3	0	0	0	0	0	0															74
40						8	17	17	6	11	5	2	2	3	0	0	0	0	0	0	0	0										71
41						16	17	14	11	12	2	0	0	0	0	0	9	0	0													81
42						15	12	24	7	12	5	4	6	4	2	2	1	1	1	0	0	0										96
43							18	22	12	9	10	2	0	0	3	0																76
44							22	18	13	5	1	1	13	4	0	2	1	1	1	0												82
45							17	21	8	11	5	5	5	5	2	2	4	2	0	0	0											87
46							22	23	14	11	11	4	3	6	3	2	3	0	2													104
47							17	15	10	8	3	3	3	7	1	1	0	0	0	0												68
48								20	14	17	7	6	5	7	3	3	2	0	2	0												86
49								14	13	11	6	4	4	4	4	4	1	0	0	2	0											67
50								24	10	12	3	10	3	2	3	2	2	2	1	1	1	0	0	0								76
51									29	0	24	4	7	3	3	6	4	4	5	5	3	2	1	1	0							101
52									20	12	8	8	3	0	2	8	1	5	1	3	4	1	3	1								80
53										16	12	4	4	7	2	2	4	3	7	0	0	0	0									61
54										20	2	6	2	2	3	2	0	1														38
55										12	7	6	4	4	1	6	0	5	1	0	0	3	0	0	0	0	0	0				49
56										29	6	9	3	5	1	(a)																53
57											24	12	7	5	7	7	3	6	6	6	6	3	1	2	3	0						98
58											25	12	3	7	3	3	3	3	2	2	2	0	0	0								65
59											29	10	3	2	6	9	4	2	6	4	4	7	0	0								86
60															1	11	4	5	7	9	8	6	4	1	6	2	0	0	0			64
61																4	10	6	8	11	10	8	9	4	6	3	6	2	0	0	0	87

[a] Escaped.

NOTE.—The females emerged the day previous to that on which the first eggs were laid and their deaths are indicated by the ends of the individual records.

approach the regularly decreasing numbers of eggs as summarized in Figure 1. During the period over which the eggs were laid the mean temperature, approximated by using the daily maxima and minima, was 66° F.

Table II gives a condensed analysis of the lives of the 61 pairs of weevils, their oviposition, and the development of their progeny:

TABLE II.—*Life history records of 61 pairs of Bruchus quadrimaculatus, together with a summary of the results of their oviposition, September 18 to October 15, 1919*

Pair No.	Date parent weevils emerged and mated.	Adult life of parent weevils.		Eggs.			Emergence of progeny.		Developmental periods of progeny.		
		Male.	Female.	Laid.	Hatched.	Produced adults.	Males.	Females.	Minimum.	Maximum.	Average.
		Days.	*Days.*	*Number.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Days.*	*Days.*	*Days.*
1	Sept. 17	11	16	99	48	32	22	10	98	171	122
2	17	13	16	76	80	71	28	26	94	153	107
3	17	11	11	74	85	73	25	29	91	177	113
4	18	16	17	115	56	43	28	22	91	164	121
5	18	10	17	106	81	65	32	37	96	181	117
6	18	10	13	106	85	81	47	39	93	179	112
7	18	12	12	85	66	45	22	16	99	188	132
8	19	14	18	93	78	62	34	24	100	193	124
9	19	12	12	71	73	68	27	21	93	167	118
10	19	14	14	82	73	65	27	26	100	205	123
11	19	14	14	93	80	68	29	34	98	132	114
12	20	11	13	81	85	79	36	28	95	188	120
13	20	13	14	84	56	50	23	19	96	153	116
14	20	9	15	85	55	46	20	19	96	154	120
15	20	14	16	85	75	64	27	27	95	181	126
16	20	13	17	81	77	72	28	30	95	167	117
17	20	14	14	97	71	55	29	24	97	172	128
18	21	14	14	95	63	55	28	24	101	216	132
19	21	11	14	71	76	66	25	22	101	175	129
20	21	14	14	90	54	42	26	12	102	216	140
21	21	16	16	96	77	72	30	39	98	165	119
22	21	11	18	87	72	63	27	28	98	164	119
23	21	17	16	61	44	34	11	10	104	173	124
24	21	13	18	75	80	75	32	24	103	179	126
25	21	17	14	77	79	68	27	25	109	179	126
26	21	15	14	81	70	63	27	24	102	194	129
27	21	15	18	92	49	41	16	22	112	170	130
28	21	15	14	91	53	43	18	21	101	153	119
29	21	13	14	91	38	35	18	14	98	164	122
30	21	11	14	82	73	70	34	23	99	167	125
31	21	14	15	74	72	65	32	16	104	194	127
32	21	16	16	78	62	45	20	15	105	184	130
33	21	13	18	95	72	59	30	26	102	198	132
34	22	15	17	99	71	67	28	38	103	168	120
35	22	15	16	73	64	60	21	23	105	158	124
36	22	10	14	88	81	53	20	27	111	179	130
37	22	9	(1)	64	47	39	18	7	103	147	121
38	22	13	16	81	73	58	24	23	102	161	122
39	22	12	13	74	84	62	25	21	102	143	121
40	22	12	18	71	89	72	32	19	102	159	122
41	22	7	15	81	70	51	23	18	108	205	128

[1] Escaped.

TABLE II.—*Life history records of 61 pairs of Bruchus quadrimaculatus, together with a summary of the results of their oviposition, September 18 to October 15, 1919*—Con.

Pair No.	Date parent weevils emerged and mated.	Adult life of parent weevils.		Eggs.			Emergence of progeny.		Developmental periods of progeny.		
		Male.	Female.	Laid.	Hatched.	Produced adults.	Males.	Females.	Minimum.	Maximum.	Average.
		Days.	*Days.*	*Number.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Days.*	*Days.*	*Days.*
42	Sept. 22	14	18	96	75	66	35	28	102	172	123
43	23	7	11	76	50	37	13	15	112	171	134
44	23	(a)	15	82	62	52	24	19	110	168	128
45	23	16	16	87	74	63	27	28	109	167	133
46	23	14	14	104	58	48	25	25	119	175	134
47	23	15	15	68	69	44	11	19	103	173	134
48	24	12	14	86	70	42	15	21	116	174	138
49	24	15	15	67	72	63	17	25	114	169	132
50	24	14	18	76	80	67	25	26	110	175	125
51	25	17	18	101	50	45	23	22	118	217	150
52	25	14	17	80	51	40	15	17	117	206	139
53	26	13	15	61	82	77	27	20	116	181	133
54	26	12	10	38	55	53	10	10	118	217	137
55	26	16	20	49	59	41	11	9	116	176	138
56	26	17	(1)	53	70	43	8	15	117	188	138
57	27	14	17	98	50	35	19	15	115	212	142
58	27	18	15	65	48	25	6	10	128	157	143
59	27	18	15	86	55	27	8	15	117	154	136
60	Oct. 1	17	16	64	80	47	17	13	131	221	162
61	2	15	17	87	68	38	17	16	131	203	162
	Average	13	15	82	67	55	23	22	.....	.....	126
	Grand total	.....	.....	5,004	.....	.....	1,429	1,320	.....	.....	.....

[a] Escaped.

NOTE.—Preoviposition period one day in all cases.

The parent weevils required from 31 to 48 days of summer weather for their own development, although they were all produced, with the exception of a few of the males and four females, from eggs laid the same day. The preoviposition period of the parent females was one day or less, as eggs recorded for the first day were deposited during the first day after emergence and mating; the females lived longer than the males; the average number of eggs per female was 82; an average of 67 per cent of all the eggs hatched, and 55 per cent produced adults. The emerged adults which resulted from these eggs were nearly equally divided as to sex, 52 per cent being males and 48 per cent females. The highest percentage of hatching of the eggs of any female was 89 per cent (No. 40) and the lowest 38 per cent (No. 29). The three females (Nos. 4, 5, and 6) which laid the largest number of eggs also had required the shortest periods for their own development—31, 33, and 33 days—which suggests that inherent vigor and favorable conditions, indicated by rapid growth, may result in unusual fecundity. In the case of the eggs which hatched, but from which no adults resulted, it was found that the larva usually died after penetrating but a short distance into the cowpea; that is, the greatest larval mortality occurred very early in life.

Emergence of the progeny began December 18, 1919, and ended May 17, 1920, a period of 152 days, over five times the duration (28 days) of the period over which the eggs were laid. The number of days required by each emerging weevil to develop from egg to adult was individually recorded, and data in Table II show the number of adults which emerged from the eggs laid by each female, as well as the average developmental period of all her adult progeny. The average time required for the development of all the males (not separately shown in the table) was a fraction of a day less than that required by the females. The shortest developmental period of the entire brood was 91 days. On the other hand, during the summer this minimum period has been observed by the writers to be about one month.

Following the supposed completion of the emergence of this series, the cowpeas were dissected and 70 live weevils in various stages of development were found. These had been developing for an average of 204 days. A live larva was dissected from the cowpeas as long as 235 days after the eggs were laid, and it is very probable that uninterrupted development on the part of the 70 forms dissected from the seeds would have resulted in developmental periods in excess of 8 months.

The following tabulation (Table III) deals with the emergence of the progeny, arranged to show the results from the daily oviposition.

TABLE III.—*Summary of the emergence resulting from eggs laid each day by 61 Bruchus quadrimaculatus*

Date laid.	Daily total of eggs.	Hatched.	Eggs producing adults.	Development period.		
				Minimum.	Maximum.	Average.
1919.		*Per cent.*	*Per cent.*	*Days.*	*Days.*	*Days.*
Sept. 18	52	81	65	91	171	*104*
19	89	75	55	93	174	*108*
20	167	84	71	91	137	*109*
21	212	77	68	95	181	*114*
22	396	77	70	93	205	*116*
23	463	77	67	98	184	*120*
24	660	74	63	97	203	*123*
25	634	70	59	110	188	*128*
26	623	70	58	105	217	*130*
27	478	66	53	110	217	*133*
28	304	54	40	115	212	*134*
29	184	56	44	115	198	*139*
30	129	50	36	103	195	*148*
Oct. 1	129	43	28	131	175	*150*
2	64	44	19	138	181	*156*
3	88	47	26	131	203	*159*
4	67	31	18	132	212	156
5	47	55	34	119	174	146
6	51	53	33	136	199	154
7	43	63	26	156	206	175
8	39	59	28	132	203	*162*
9	30	50	37	155	221	175
10	18	67	33	151	186	167
11	9	33	33	163	167	165
12	15	40	7	160	160	160
13	5	40	0	.....	.....	.....
14	6	0	0	.....	.....	.....
15	2	0	0	.....	.....	.....
Total or average	5,004	67	55	.....	.....	126

NOTE.—Temperatures during egg-laying period are included in Table I.

During the first two-thirds of the laying period, indicated in Table III, the constant increase in the length of the average developmental periods of weevils from eggs laid on successive days is a good illustration of the effect of the approach of winter. Our understanding of heat unit values at different temperatures with respect to the rapidity of development of *Bruchus quadrimaculatus* is not equal to the task of correlating these progressively lengthened periods with the thermal environment by an analysis of the thermograph records. There are, however, certain points which should be briefly discussed.

That the mortality of young larvæ is higher than that of older individuals has been noted, and it is reasonable to suppose that they are very responsive to temperature changes, because they are not deeply buried in the seeds. In the fall, young larvæ hatched on successive days are usually exposed to optimum temperatures for progressively shorter periods, and a difference of a few days of warmth at the beginning of the development of two groups of weevils at that season may be magnified to a greater difference in their developmental periods. Thus, in the case of the eggs deposited September 18 and 19, a difference of one day in the duration of the exposure of the eggs and young larvæ to warm weather at the beginning of the period of growth resulted in a difference of four days in the average time required for all the weevils from these eggs to emerge, as shown in the last column of the table. Likewise, in the case of eggs deposited September 18 and 27 (the latter being the date of a cold storm), a difference of 9 days of warmth at the beginning of the life of the weevils seems to be responsible for the ultimate difference of 29 days in the average period required for emergence.

Toward the end of the egg laying (October 4 to 15) the small number of individuals concerned probably tended to make the last few average developmental periods inconsistent with the constant increase of the preceding ones.

The mean monthly temperatures in the laboratory during the period covered by the development of the brood were as follows: September (18 to 30), 68.5° F.; October, 61.5°; November, 58°; December, 61°; January, 59.5°; February, 59.5°; March, 60°; April, 63°; May, 64°.

Considerable variations were noted in the length of the periods required for the development of individual weevils from eggs laid by the same female on the same day. An example illustrating this point is given in Table IV, which also shows one of the most nearly typical egg records, including the diminishing number and vitality of the eggs toward the end of the laying. (See also fig. 1.) The results of the oviposition of all the females, similarly worked out, are abstracted in Table II.

Data used to prepare figure 1 show that there was a greater percentage of hatching of eggs laid early in the life of a female than toward the end, and an even more marked preponderance of the earlier eggs produced adults. Figure 1 applies to all the eggs of all the females, consolidated on the basis of the individual reproductive life. That is, the first day's eggs of all the 61 females are consolidated under "laying day" 1, etc. Only three females extended their oviposition over 15 laying days. Dates, as well as days on which individuals laid no eggs, are not considered, the 61 egg records of the series being in effect telescoped so that their beginnings are coincidental. The tendency toward heavy oviposition during the first few laying days and the decreasing number and fertility of the eggs as the females approached death are shown. The higher degree of vitality which characterizes the earlier eggs of an indi-

vidual seems to extend beyond the ability of a large percentage of them to hatch and to lend vigor to the growing progeny, promoting a higher percentage of emergence. To avoid complicating figure 1, this varying difference between percentage of hatching and percentage of emergence is not illustrated, the former being omitted.

TABLE IV.—*Minimum, maximum, and average length of the developmental periods of adults resulting from eggs laid by one female (No. 48)*

Date of oviposition.	Eggs laid.	Eggs hatched.	Adults emerged.			Length of developmental period.								
						Total.			Males.			Females.		
			♂	♀	Total.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.
						Days.	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*
Sept. 25	20	14	5	9	14	116	155	130	116	132	122	116	155	134
26	14	12	3	3	6	121	163	143	121	158	140	123	163	145
27	17	13	2	4	6	117	174	149	117	158	138	133	174	155
28	7	5	3	2	5	128	152	138	128	152	139	128	146	137
29	6	5	1	2	3	122	135	127	135	135	135	122	123	123
30[a]	5	5	1	0	1	161	161	161	161	161	161	0	0	0
Oct. 1	7	2	0	1	1	158	158	158	0	0	0	158	158	158
2	3	2	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....
3	3	1	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....
4	1	1	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....
6	2	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total or average	86	60	15	21	36	116	174	138	116	161	135	116	174	140

[a] One male was dissected alive from the cowpeas after 218 days of development.

A study of all the data, which are here published only in abstract, suggests that the age of females affects the *rapidity* of the development of their progeny. For example, the average developmental periods of all the weevils which resulted from eggs laid October 7 are roughly proportional to the age of the parent females on that date. Females Nos. 51, 52, 57, 60, and 61 were respectively 12, 12, 10, 6, and 5 days old on October 7; and the average developmental periods of all the progeny from the eggs laid that day by these weevils were 206, 206, 172, 167, and 165 days. This effect is supported by other evidence in the complete analyses, which, however, are too extensive to be published.

It is of course generally recognized that the moisture content of beans and other seeds influences the development of insects feeding in them. The moisture content of the cowpeas used in these experiments was not determined, but they were kept under uniform conditions which would tend to cause the percentage of moisture to change equally in all the seeds. Possibly dryness was responsible for some larval mortality, but figure 1 shows that the principal factor influencing mortality of immature forms is the age of the mother at the time of oviposition.

CONCLUSIONS

The results of this study indicate that the average lengths of the developmental periods of larvæ of *Bruchus quadrimaculatus* which hatch from eggs laid on successive days in the fall tend to be inversely proportional to the duration of the exposure of the embryos and young larvæ to warm weather.

The age of a female weevil at the time of laying a given day's batch of eggs influences (1) the number of eggs in the batch, and (2) the vitality of the eggs, as indicated by (*a*) the percentage of hatching, (*b*) the ability of the resultant larvæ to become adults, and (*c*) the average duration of the developmental periods of the progeny.

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WASHINGTON : GOVERNMENT PRINTING OFFICE : 1923